HEMATOGENOUS AND OBSTRUCTIVE ICTERUS.

Experimental Studies by Means of the Eck Fistula.*

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The view that bile pigments are derived solely from the hemoglobin of the blood corpuscles, and that this change under physiological conditions is brought about only in the liver, is generally accepted at present. It is possible that the evidence submitted in this and the following paper may give rise to doubts as to whether the mechanism of bile pigment formation can be explained in this simple fashion. The older work on the subject is fully presented by Stadelmann and Minkowski, and will therefore not be discussed here. The liver is the excretory organ for bile pigments, and the introduction of bile pigment intravenously (Stadelmann) is followed by a sharp rise in the output of the bile pigments in the bile. The same thing occurs when hemin (Brügsch and Yoshimoto) or hemoglobin (Tarchanoff) is injected intravenously, or when substances are administered which lake the hemoglobin in the vessels. But this does not prove that bile pigments can be formed from no other material than hemoglobin.

It is admitted by most workers that under certain conditions when blood escapes into the tissues a substance (hematoidin) may be formed after a considerable time which is chemically identical with bilirubin. The same sequence may occur in the pleural cavities (Guillain and Troissier). This reaction is thought to be of little importance in explaining the general formation of bile pigments and the pigmentation of icterus.

It seemed of interest to study the various types of icterus by means of the Eck fistula which shunts the portal blood around the

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liver and limits its blood supply essentially to the arterial system. As an example of the first type of icterus in which large amounts of hemoglobin are set free in the circulation, the introduction of laked red blood cells intravenously is less confusing than hemolysis produced *in vivo* by immune sera, toluylendiamin, etc. It could be argued that the intoxication produced by these various poisons (serum or drugs) might modify the normal reaction of the organs and that deductions drawn from such experiments might be unjustifiable. As an example of the second type of hematogenous icterus, the liver was injured severely by chloroform anesthesia, which is known to cause liver cell necrosis. It is thought that this cell injury and necrosis may cause primarily capillary obstruction in the bile canaliculi and secondarily icterus. Simple obstructive jaundice was produced by ligating and cutting the common bile duct. These three procedures represent fairly accurately the various common types of icterus, the first two usually grouped under the term hematogenous and the last designated obstructive. Most writers say that this is a forced classification, and that all icterus is in reality hepatogenous and therefore obstructive, whether affecting the finer bile capillaries or the larger ducts.

METHOD.

Dogs were used in all the experiments, and were usually strong, healthy females. Unless otherwise stated, the urine was obtained by catheter. Huppert's test for bile pigments was used in examining concentrated highly colored urine of the dog. A measured amount of filtered urine was precipitated with calcium chloride in a solution made alkaline by sodium carbonate. The precipitate was thrown upon a filter, washed with water, and dissolved in hot acid alcohol. By concentrating the acid alcohol to a fixed volume (5 or 10 c.c.) the green color could be compared easily and a rough estimate of the relative amount of bile pigment readily made. The Eck fistula operation was that devised by Jeger, who joins the portal vein and the vena cava by means of clamps, as is done in the usual operation for lateral entero-enterostomy. Active diuresis was usually secured by intravenous injection of normal saline solution (0.9 per cent.) in varying amounts.

EXPERIMENTAL OBSERVATIONS.

HEMOGLOBIN INJECTED INTRAVENOUSLY INTO A NORMAL DOG.

Dog C–80.—Large mongrel, female; weight, 28 pounds.

June 3, 9 A. M. Dog in excellent condition. Urine negative for bile pigments. 11 A. M. Ether anesthesia begun. 11.10 A. M. Red blood corpus-

cles (25 c.c.) washed with salt solution and laked with an equal volume of distilled water were injected into the jugular vein. Following this 600 c.c. of 0.9 per cent. salt solution were introduced slowly intravenously. 11.20 A. M. Urine (8 c.c.) contains hemoglobin but no bile pigments. 11.50 A. M. Urine is deep red in color. 10 c.c. contained much hemoglobin but no bile pigments. 12.10 P. M. Urine (11 c.c.) is dark red in color. Hemoglobin very abundant. Bile pigments are negative. 12.40 P. M. Urine is dark red in color and contains much hemoglobin. Bile pigments are positive. 2.10 P. M. Urine is dark red in color. Hemoglobin is abundant and bile pigments are strongly positive. 3 P. M. Urine is dark red in color. Hemoglobin is abundant. Bile pigments are positive. 5.30 P. M. Urine amber colored. Bile pigments and hemoglobin are absent.

June 4, 9 A. M. Dog is in excellent condition. Urine is clear amber colored. Bile pigments and hemoglobin are negative. The dog was used later for a similar experiment after the liver had been excluded from the circulation, and the reaction in general was found to be the same.

Dog C-83.—Black mongrel, female; weight, 17 pounds.

May 28, 9.40 A. M. Dog in excellent condition. Urine is dark amber colored. It contains no bile pigments and no hemoglobin. Ether anesthesia and bleeding from the external jugular vein. The red blood cells were obtained by centrifugalization, washed, and finally 28 c.c. of washed red blood cells were laked with an equal quantity of distilled water and injected intravenously, followed by 600 c.c. of 0.9 per cent. salt solution. 10.40 A. M. Urine is dark reddish brown. Hemoglobin is abundant and bile pigments are present. 11.40 A. M. Urine is similar in appearance. Hemoglobin is abundant and bile pigments are present in small amounts. 12.40 P. M. Urine is dark reddish brown. Hemoglobin is abundant and bile pigments are positive. 2.30 P. M. Urine is dark reddish brown. Bile pigments and hemoglobin as in the previous specimen. 4.30 P. M. Bile pigments are present and hemoglobin is still abundant.

May 29, 9 A. M. Urine is concentrated and dark amber colored. Hemoglobin is absent and the bile pigment test is suspicious but not positive.

May 30, 9 A. M. Dog is well. Urine is clear and contains no bile pigments and no hemoglobin. The dog was used later for a similar experiment after partial exclusion of the liver, and the reaction was found to be practically identical.

Dog C-53.—Bull dog, female; weight, 27 pounds.

May 26, 9 A. M. Urine is clear amber colored and contains no bile pigments. 9.30 A. M. Blood was taken from the external jugular, centrifugalized, and the red blood cells were washed with salt solution. 30 c.c. of these red blood cells were then laked with an equal volume of distilled water and injected intravenously.

9.45 A. M. Injection of red blood cells is followed by the injection of 500 c.c. of 0.9 per cent. salt solution. 11 A. M. Urine (20 c.c.) is a turbid, red color and contains no bile pigments but abundant hemoglobin. 11.30 A. M. Urine is a turbid red color. 10 c.c. give positive tests for bile pigments, and hemoglobin is abundant. 1 P. M. Urine is dark red in color. 10 c.c. give positive bile pigment tests and abundant hemoglobin. 3 P. M. Urine is a reddish color. 10 c.c. give a positive suspicious test for bile pigments and con-

tain hemoglobin. 4.45 P. M. Urine is amber colored and contains no hemoglobin. 10 c.c. give a positive suspicious test for bile pigments.

May 27, 9 A. M. Urine is clear amber colored. Negative for hemoglobin and bile pigments.

HEMOGLOBIN INJECTED INTRAVENOUSLY INTO AN ECK FISTULA DOG AFTER SPLENECTOMY.

Dog C-79.—Mongrel, female; weight, 22 pounds.

May 25, 11 A. M. Dog is in excellent condition. Ether anesthesia and operation as usual with the production of an Eck fistula and extirpation of the spleen.

May 26. Dog is making an excellent recovery.

June 1, 9.40 A. M. Ether anesthesia and blood drawn from the external jugular vein. The red blood cells were obtained and washed in the usual way. 500 c.c. of 0.9 per cent. salt solution were given intravenously. 10 A. M. 30 c.c. of red blood cells were laked with an equal volume of distilled water and introduced intravenously. Urine is clear amber colored, and is negative for bile pigments (10 c.c.). 10.10 A. M. Urine has a slight reddish tinge and contains hemoglobin. 20 c.c. are negative for bile pigments. 10.25 A. M. Urine is red in color. 30 c.c. contain hemoglobin and no bile pigments. 11.00 A. M. Clear reddish urine. 70 c.c. contain hemoglobin and give a positive test for bile pigments. 11.30 A. M. Urine is a clear reddish color. 130 c.c. contain hemoglobin, and bile pigments are positive. 12.30 P. M. Clear reddish urine. 60 c.c. contain hemoglobin. 10 c.c. give a positive test for bile pigments. 3 P. M. 90 c.c. of clear urine is free from hemoglobin. 20 c.c. give a positive test for bile pigments. 5.30 P. M. 40 c.c. of clear amber colored urine is negative for bile pigments and hemoglobin.

June 2, A. M. Clear amber colored urine. 20 c.c. are negative for bile pigments and hemoglobin.

June 9. Dog has convulsions and died after a few minutes.

Autopsy.—Performed at once. The heart, lungs, and thorax are negative. The stomach, intestine, and pancreas are normal. The liver is rather small and somewhat pigmented. Bile can be squeezed from the gall bladder into the duodenum. The Eck fistula is perfect. The ligature on the portal vein is just at the hilum of the liver and there are no collaterals into the portal vein above this point. There are very few adhesions about the site of operation. The kidneys are large and on removal of the capsule show a mottled cortex, pale, in general, but specked with pink and reddish areas as well as ecchymoses. The cortex is wide and rather irregular on section.

Microscopical Examination.—An acute suppurative nephritis is present. There are great numbers of pus cells in the stroma as well as in the tubules. Examination of the urine at autopsy shows an enormous amount of albumin. The liver is practically normal, except for a certain amount of pigmentation of the Kupfer cells, which undoubtedly is associated with the injection of hemoglobin. There are a few pus cells in the capillaries but no liver cell injury.

HEMOGLOBIN INJECTED INTRAVENOUSLY AFTER SPLENECTOMY.

Dog C-92.—Small mongrel, female; weight, 14 pounds.

June 14. Ether anesthesia and extirpation of the spleen, which was normal.

June 21. Animal is in excellent condition. 9.30 A. M. 100 c.c. of urine are negative for hemoglobin, and 50 c.c. are negative for bile pigments. 10 A. M. Ether anesthesia and blood removed from the jugular vein. The red blood cells (25 c.c.) were washed and laked with distilled water in the usual way. 10.15 A. M. Dog is given 300 c.c. of 0.9 per cent. salt solution intravenously. 10.30 A. M. Urine (10 c.c.) gives a negative test for bile pigments. The laked red blood cells are introduced intravenously and followed by 350 c.c. of 0.9 per cent. salt solution. Hemoglobin appears in the urine in seven minutes. Ether anesthesia discontinued. 11 A. M. Urine (25 c.c.) is deep claret red in color, and shows large amounts of hemoglobin. Bile pigment tests are negative. 11.30 A. M. Urine is dark red in color. 11 c.c. were negative for bile pigments and contained large amounts of hemoglobin. 12 M. Urine is dark red and rather turbid. 15 c.c. gave a faintly positive test for bile pigments, and hemoglobin was conspicuous. 12.30 P. M. Urine is dark red in color. 15 c.c. show a large amount of hemoglobin, and 10 c.c. give a positive test for bile pigment. 1 P. M. Urine is dark claret red. 12 c.c. contain much hemoglobin and give a strongly positive test for bile pigments. 3 P. M. Urine (50 c.c.) is pale red in color, and contains hemoglobin. 15 c.c. give a strong positive test for bile pigments. 5 P. M. Urine (100 c.c.) is pale red in color, contains hemoglobin, and gives a strong test for bile pigments.

June 22, 9 A. M. Urine (25 c.c.) contains no hemoglobin and gives a strong test for bile pigments.

June 23. Urine (20 c.c.) gives a very faint test for bile pigments.

TABLE I.

Hemoglobin Injected Intravenously.

No. of dog.	Condition.	Laked blood corpuscles.	Hemoglobin in urine.		Bile in urine.	
			Beginning.	End.	Beginning.	End.
C–80	Normal	25 c.c.	10 min.	6 hrs.	1½ hrs.	6 hrs.
C–83	Normal	25 c.c.	5 ± min.	8 + hrs.	1 hr.	24 hrs.
C–53	Normal	30 c.c.	5 ± min.	7 hrs.	1¾ hrs.	7 hrs.
C–79	Eck fistula and extirpation of spleen	30 c.c.	6 min.	5 hrs.	1 hr.	7½ hrs.
C–92	Extirpation of spleen	25 c.c.	7 min.	7 + hrs.	1½ hrs.	48 hrs.

In our preliminary experiments an effort was made to study the formation and excretion of bile pigments following an injection of laked red corpuscles into the peritoneal cavity. The reaction following this procedure was not satisfactory for our needs in the later experiments with liver exclusion, as the initial appearance of hemoglobin and bile pigments in the urine was often greatly delayed. In the same way the action of several poisons was tried, the object being a rapid hemolysis *in vivo* with a study of the pigments in the urine. There is often considerable delay in this reaction, and it was

thought that objections could be raised to such experiments with the introduction of toxic substances which may have manifold actions on various organs and tissues, besides the desired action of hemolysis.

The introduction of red corpuscles obtained from the same dog, washed and laked with distilled water, seemed to offer least objections and gave the most satisfactory results. This simulates the explosive type of paroxysmal hemolysis which is seen in patients with paroxysmal hemoglobinuria and in blackwater fever. In such cases the appearance of pigments in the urine and body fluids is familiar and similar to our experiments with dogs.

In the type experiments cited and tabulated above it is found that after intravenous injection of hemoglobin the pigments appear in the urine after a constant space of time: hemoglobin after five to ten minutes, and bile pigments in one to one and a half hours. The duration of excretion varies considerably in normal dogs, but the hemoglobin disappears before the bile pigments, usually within twelve hours. The Eck fistula dogs react exactly as do the normal dogs and this fact is worthy of consideration. It is known from the work of Burton-Opitz that about three fourths of the blood flowing through the liver comes by way of the portal vein, the remainder being furnished by the hepatic artery. If the elaboration of bile pigments is effected solely by the liver epithelium, it is striking that the time of appearance of bile pigments in the urine is not delayed by an Eck fistula, which greatly lowers the blood volume passing through the liver. This point will be touched upon later, and it will be seen that the bile pigments appear in the usual time after the injection of hemoglobin when an Eck fistula is combined with ligation of the hepatic artery. This prevents active hepatic circulation and the dogs die from hepatic insufficiency.

NORMAL DOG. CHLOROFORM ANESTHESIA. JAUNDICE.

Dog C-19.—Large young mongrel, female; weight, 23 pounds.

January 30, 11 A. M. Chloroform anesthesia for 2¾ hours was given at the same time and under similar conditions with dog C-20. Urine obtained at the end of anesthesia was concentrated (15 c.c.) and negative for bile pigments. 7 P. M. Urine is negative for bile pigments. January 31, 10 A. M. Urine (250 c.c.) is orange yellow in color. Bile pigments are present in large amounts. Dog is sick and weak.

February 1, 3 P. M. Urine (70 c.c.) is clear orange yellow. Bile pigments

are present in large amounts. Dog is slightly improved. No vomiting. There is slight jaundice of the mucous membranes and scleræ.

February 2, 10 A. M. Urine (400 c.c.) obtained from under the cage is a deep orange yellow and gives only a faintly positive test for bile pigments. Dog is improving.

February 3. Dog is in fairly good condition. Jaundice has cleared up. Urine is concentrated and highly colored and gives a faint trace of bile pigments.

February 4. Dog has lost considerable weight, 17¾ pounds, but seems in good condition.

ECK FISTULA. CHLOROFORM ANESTHESIA. JAUNDICE.

Dog C–21.—Mongrel, female; weight, about 35 pounds.

January 4. Ether anesthesia and production of an Eck fistula in the usual manner.

January 30, 11 A. M. Chloroform anesthesia for 2¾ hours. The experiment was conducted at the same time and under the same conditions with dog C-19. Urine obtained before anesthesia contains no bile pigments. No urine was obtained from the bladder at the end of the anesthesia. 7 P. M. Urine obtained in the cage contains no bile pigment. January 31, 10 A. M. Dog is quiet and does not vomit. Urine, collected under a clean cage, is highly colored and gives a positive test for bile pigments which, however, are not very abundant.

February 1. Dog does not seem very sick and will eat. Jaundice is well marked in the skin, mucous membranes, and scleræ. Urine is clear amber colored, and in parallel tests with dog C-19 contains the same amount of pigments in the same volume of urine.

February 2. Dog refuses food. Urine obtained from under the cage gives a strong pigment test, much more so than an equal amount from dog C–19.

February 3. Dog is in poor condition. Urine obtained from below the cage gives a positive test for bile pigments.

February 4. Dog is improving and eats well. Bile pigments are suspicious in the urine and the test is not positive.

February 20. Dog is found in convulsions. Given ether and bled to death.

Autopsy.—The heart contains one large filaria. The lungs, spleen, kidneys, pancreas, and intestines are normal. The stomach contains a large amount of food including bones. The abdominal cavity shows only a few adhesions about the site of operation between the liver and duodenum. The fistula shows a perfect result with a large opening. The ligature on the portal vein obliterates the lumen. Just above the ligature there is a tiny thread-like collateral opening into the portal vein above the ligature, communicating with the gastrohepatic omentum through which a very small amount of blood could get into the portal vein.

The liver is small and of a greenish yellow color. The lobules are conspicuous and the organ cuts with difficulty. The centers are brownish and the edges of the lobules are yellow.

Microscopical Examination.—Sections show a great deal of fat throughout the liver lobule. The lobules are rather small. In the centers of the lobules one finds little balls and clumps of yellow granular pigment, undoubtedly the

result of the liver injury due to chloroform followed by healing which has been practically complete. In these areas one finds also a few wandering mononuclear cells.

TABLE II.

Chloroform Anesthesia. Jaundice.

Time.	Normal dog (*C*-19).		Eck fistula dog (*C*-21).	
	Urine in c.c.	Bile pigment.	Urine in c.c.	Bile pigment.
Jan. 30	20	0	10	0
11:00 A.M. to 1:45 P. M.	Chloroform anesthesia for $2\frac{3}{4}$ hrs.			
2:00 P.M.	15	0	0	—
7:00 P. M.	15	0	20 ±	0
Jan. 31	10	++++	10	++
Feb. 1	10	++++	10	++++
Feb. 2	10	+(?)	10	+++
Feb. 3	10	+(?)	10	++
Feb. 5	—	—	12	+(?)

The two preceding experiments are types giving a fair picture of the result of chloroform anesthesia. The experiments were done at the same time and under similar conditions for comparison. It is known (Whipple and Sperry) that the injury following chloroform poisoning is practically limited to the liver where a central necrosis is the characteristic lesion. This lesion is produced in dogs with Eck fistulæ, but is much less easily produced than in normal dogs, and usually of a more limited extent. In other words, the liver of an Eck fistula dog is resistant to chloroform injury.

Fischler and Bardach have shown the same resistance to phosphorus poisoning in Eck fistula dogs and claim that this speaks against the specificity of the toxic action of phosphorus upon the liver. Surely there can be nothing more specific than the action of chloroform on the liver, yet the injurious action is less evident in a dog with an Eck fistula. It is not difficult to understand why this injury is less evident, when we consider that the portal blood is cut out of the liver lobules, where the circulation must be relatively sluggish, and consequently the parenchyma cells are brought into contact with only a fractional part of the injurious agent, as compared with the normal circulatory volume. The liver cells are somewhat atrophied and presumably not as active as normally, a condition which might modify their susceptibility to a given drug.

In these experiments we may assume with certainty that the same

lesion was present in both, namely, a central necrosis of each liver lobule. Of the extent of this necrosis we cannot be sure, as no tissue was removed for examination. Icterus appeared in both animals, and bile pigments in the urine. The bile pigments in the urine of the Eck fistula dog showed some delay in appearance but persisted longer, and we may explain the long duration by a necessary delay in the healing and reparative process which always follows this liver injury. Of necessity the normal liver with its rich blood supply would repair and regenerate the liver cells more rapidly than the liver supplied with blood only by the hepatic artery. On the second day, the time of maximum liver injury and intoxication, the amount of bile pigments in the urine was the same in the two dogs.

How can we explain this parallelism in bile pigmentation of the urine, as it is clear that dogs with Eck fistulæ secrete less bile than normal dogs? It is not enough to say that the liver necrosis and injury cause a capillary obstruction in the biliary tree, for the amounts of bile pigment excreted are not similar in these two conditions. We must refer to the work of Joannovics and Pick who showed in poisoned livers the presence of a powerful hemolysin. Granting the presence of such a hemolysin, it is quite simple to explain this equal formation of bile pigments.

We do not claim that chloroform anesthesia for a unit period will always cause a unit grade of icterus and pigmentation of the urine. As a rule, the liver injury will be much less in an Eck fistula dog, and therefore the bile pigment excretion in the urine somewhat less than in a normal dog. But it is evident that the maximum pigment excretion is similar in the two conditions and happens on the second day after the injury. Fischler and Bardach observed less intense icterus with phosphorus poisoning in Eck fistula dogs as compared with normal controls, and one may expect a certain degree of parallelism between the extent of the liver injury and the escape of bile pigment from the liver into the general circulation.

ECK FISTULA. OBSTRUCTIVE JAUNDICE.

Dog C–10.—Large bull dog, female.

February 17. Ether anesthesia and Eck fistula produced as usual. The animal recovered rapidly and was in excellent health during the following month.

April 30. Ether anesthesia and operation. The common bile duct was isolated, ligated doubly, and cut between ligatures.

May 1. Dog in excellent condition. No apparent icterus of the sclerotics. Urine is clear amber colored. 5 c.c. give a positive bile pigment test.

May 2. Feces are clay colored. Urine is concentrated and gives a strong test for bile pigments.

May 3. Mucous membranes show a definite icteric tinge. Urine is highly colored but contains no hemoglobin. Bile pigments are abundant.

May 4 and 5. Condition is unchanged.

May 6. Dog is very active. Skin and mucous membranes all show a definite lemon color. Urine contains a large amount of bile pigment.

May 7, 9, and 11. Condition unchanged. The urine is constantly positive for bile pigments.

May 13 to 16. Condition excellent. Feces clay colored. Urine gives a strong bile pigment test.

May 18 to 20. Skin and mucous membranes are definitely jaundiced. Feces are clay colored. Bile pigments are present in the urine.

May 22. Condition unchanged. 5 c.c. of urine give a positive test for bile pigment.

May 24 to 28. Condition is unchanged. Dog is active and shows the same grade of icterus. Weight, 33 pounds. 5 c.c. of urine give a positive test for bile pigments. Feces are clay colored. Urobilin and stercobilin are absent.

May 30. Condition is unchanged. Bile pigments in 5 c.c. of urine are positive. Urobilin and stercobilin tests are negative.

June 1 to 5. Condition is unchanged.

June 7 to 9. Icteroid color is constantly present. 5 c.c. of urine give positive tests for bile pigments.

June 11. Dog is in good condition; weight, 34 pounds. 5 c.c. of urine give a positive test for bile pigments.

June 14 to 27. Condition remains unchanged. Urine tested every two days shows positive tests for bile pigments in 5 c.c.

June 29 to July 7. Condition is unchanged. The jaundice seems to be clearing slightly in the skin and conjunctivæ. Urine, however, still gives positive bile pigment tests. Feces are clay colored.

July 11 to 19. Urine contains a little less bile pigment. 10 c.c. give a positive test of the usual intensity.

July 22. Weight, 32 pounds. Feces are clay colored. Stercobilin and urobilin tests are positive. The urine gives a positive bile pigment test in 10 c.c.

July 24. Dog in excellent condition. Weight, 30 pounds. Ether anesthesia and bled to death. Urine obtained from the bladder gives a positive test for bile pigments. 35 gm. of omental fat give a positive test for bile pigments. 50 c.c. of serum give a strongly positive test for bile pigment.

Autopsy.—Performed at once. The subcutaneous tissue, mucous membranes, and scleræ show a definite icteric tint. The subcutaneous fat throughout is not abundant but is definitely pigmented. The thorax, lungs, heart, spleen, pancreas, and kidneys are normal. The peritoneal cavity shows some old adhesions around the sites of operation. The liver is small, rather tough on section, and deep brown with a definite greenish tint. The lobulation is regular. The gall bladder is enlarged and tense. Its wall is definitely thickened.

The common bile duct is dilated and thickened, measuring about 2 cm. in

diameter. The intestines are cut away and opened. They contained pasty feces showing signs of bile pigmentation. The duodenum is opened carefully and the papilla opened and washed out. Much pressure upon the distended gall bladder caused the oozing of a tiny drop of bile, showing that communication had been established in some way and that bile was escaping very slowly into the intestinal loop. Dissecting up from the papilla one came to the ligature which was lying in the wall of the duct which is thickened at this point. The other ligature was embedded in adhesions lying in the wall of the dilated duct above, a distance of about 1 cm., the distance which the cut ends usually separate at the time of section. Between these two ends is a small smooth lined cavity containing bile-stained material which communicated by a tiny tortuous fistula with the lower end of the dilated common duct, and again with the upper end of the distal portion of the common duct. The common duct above the ligature is dilated and thickened, lined with intact mucosa, and contains tenacious dark green bile. The fact that the bile duct can establish a lumen in this peculiar manner where it has been doubly ligated and cut shows the necessity of careful autopsies in such experiments.

The Eck fistula was perfect. The ligature on the portal vein above the fistula obliterated the lumen completely. Just above this ligature one tiny thread-like collateral communicated with the gastrohepatic omentum, and through this collateral a very small amount of blood could get into the portal vein.

Microscopical Examination.—The liver shows normal parenchyma cells except for atrophy and a trifling amount of fatty change. The bile capillaries are conspicuous in places and contain yellow colloid material. The connective tissue about the bile ducts and portal spaces is increased, and in these areas one sees some bile duct proliferation. In addition mononuclear and polymorphonuclear cells are found in these areas, some of the large mononuclear cells being filled with pigment. The kidneys are normal.

The preceding experiment (dog *C*–10) deserves especial notice as the animal was in excellent condition during the entire observation, and tests for the amount of bile pigment were made daily during the first month and every second day until the end of the experiment, a period of twelve weeks. The Eck fistula was produced on February 17 and the dog followed carefully in connection with another experiment. The common bile duct was ligated and cut on April 30, and after this date bile pigments were constantly present in the urine in uniform amounts. On the third day later one could see a faint icteric coloring of the mucous membranes and after a week the jaundice became constant and remained obvious to casual observation even in the skin, mucous membranes, and scleræ. The weight showed a fairly constant level. During the last two weeks the bile pigments lessened in amount and about double the amount of urine was needed to give the same green color in Huppert's test.

Stercobilin and urobilin, which had been negative before this, now were present in the feces and urine, and it seemed clear that some bile pigments must be escaping into the alimentary canal. We were at a loss to understand this until at autopsy a tiny fistulous tract was found between the separated ends of the common bile duct, permitting the escape of a little bile into the duodenum. The obstruction was still obvious and all the passages and the gall bladder were dilated and thickened.

The observation that the common duct can establish its continuity after double ligation, cutting, and separation of the ends is of interest in connection with experimental work on obstructive jaundice and calls for careful post-mortem examination. A second dog (*C*–55) showed an identical result about one month after the operation. The following experiments confirm this in every particular. Extirpation of the spleen does not modify the picture.

ECK FISTULA. EXTIRPATION OF THE SPLEEN. OBSTRUCTIVE JAUNDICE.

Dog C–90.—Small mongrel, female; weight, 10½ pounds.

June 7, 9 A. M. Urine is clear amber colored and contains no bile pigment. Ether anesthesia and Eck fistula produced. The common bile duct is isolated and cut between the ligatures. The spleen is extirpated.

June 8. Urine (5 c.c.) contains bile pigment.

June 9. Urine contains considerable bile pigment.

June 10. The mucous membranes and skin show faint yellowish tingeing. Animal refuses food and is fed by stomach tube. Bile pigments are constantly present in the urine. Feces are clay colored. Urobilin and stercobilin tests are negative.

June 12. Condition continued the same. Bile pigments are present in considerable amounts.

June 13. Weight, 9¾ pounds.

June 14 to 16. Urine contains large amounts of bile pigments in 5 c.c.

June 17. Stools are clay colored and semifluid. Urobilin and stercobilin tests are negative. Bile pigments are abundant in the urine.

July 19 to 22. Condition remains unchanged. Bile pigments are present constantly in the urine; the skin and mucous membranes show definite jaundice.

July 23. Dog is rather weak and vomits after being fed by stomach tube. 5 P. M. Ether anesthesia and bleeding from the carotid. 25 c.c. of blood plasma give a strong bile pigment test.

Autopsy.—Performed at once. The subcutaneous fat has undergone advanced atrophy. All the tissues show the bile tingeing. The heart is normal. The lungs show scattered patches of bronchopneumonia from 1 to 3 cm. in diameter. Stomach, pancreas, and intestine are normal. The feces in the intestine are clay colored and negative for stercobilin.

The Eck fistula is patent and the portal vein above the fistula is completely occluded by the ligature above it. Careful dissection shows not the smallest collateral. The gall bladder and common duct are greatly dilated, but only slightly thickened, and they contain dark, slimy bile. The cut ends of the common bile ducts are separated a distance of about 2 cm.

Microscopical Examination.—The lungs show areas of bronchopneumonia with beginning abscess formation. There is a slight increase in connective tissue about the portal spaces of the liver. The edges of the lobules show normal liver parenchyma. The center of the lobule shows some congestion and a slight amount of atrophy of the parenchyma. In addition there are large phagocytes containing yellow granular pigment, and in places the bile canaliculi are dilated with a greenish colloid substance.

ECK FISTULA. OBSTRUCTIVE JAUNDICE. CHLOROFORM POISONING.

Dog C–40.—Mongrel, female; weight, 16 pounds.

April 2. Ether anesthesia and Eck fistula produced as usual. The common bile duct was ligated in two places and cut between the ligatures. Urine obtained before the operation was clear amber colored and contained no bile pigments.

April 3. Dog is in good condition and eats well. Urine is pale amber colored and contains bile pigments.

April 4. Dog refused food. Urine is concentrated and dark reddish brown in color. It contains bile pigments.

April 5. Bile pigments are strongly positive in the urine.

April 6. Dog refuses food and is given milk by stomach tube. Urine is concentrated and contains bile pigments.

April 7. Condition slightly improved. Urine contains bile pigments.

April 8 and 9. Wound is clean and animal is slowly improving. The urine gives a positive test for bile pigments.

April 11 and 12. Condition remains unchanged. The scleræ and skin show definite lemon yellow jaundice pigmentation.

April 13, 9.30 A. M. Dog is given intraperitoneally 30 c.c. of washed red blood corpuscles laked in distilled water. The urine from 9.30 A. M. to 4 P. M. showed a constant amount of bile pigments, but after this time it showed an increasing amount of bile pigments, tests being made in each instance on 2 c.c. of urine and under similar conditions.

April 14. Urine contains a large amount of bile pigments.

April 15. Urine (2 c.c.) gives a strong test for bile pigments. Hemoglobin has been negative in the urine during the past two days.

April 16. Bile pigments have diminished to the normal level.

April 17. Dog shows definite bile tingeing of the mucous membranes and the skin. Feces are clay colored and bile pigments have been constantly present in the urine.

April 19. Dog is improving. Sclerotics and mucous membranes are definitely pigmented. 4 c.c. of urine give a positive bile pigment test.

April 20. The experiment performed on April 13 was repeated and the findings were identical.

April 23. Dog will not eat and is given food by stomach tube. Urine is

clear amber in color. 4 c.c. give a definite test for bile pigment. Chloroform anesthesia for two hours.

April 25. Dog is quite sick and will not eat; has lost a great deal of weight. Weight, 10 pounds. The urine contains bile pigments. Ether anesthesia and bleeding from carotid.

Autopsy.—Performed at once. The blood plasma gives a strong positive test for bile pigments. The thorax, heart, and lungs are normal. The spleen, pancreas, stomach, and intestines are normal. The kidneys show tiny linear abscesses through the cortex and pyramids, outlined with thin reddish zones of hemorrhage. The bladder is normal. The peritoneum, particularly the omentum, shows a greenish yellow staining due to the injection of the red blood cells. Fat is practically absent but the icteric tinge is evident in all the tissues.

The liver is a deep olive green and tough on section. The adhesions about the site of operation are quite dense and firm. The gall bladder and common bile duct contain dark green, slimy bile. They are all distended, tense, and show thickened walls, but not to the extent noted in the dog with simple obstructive jaundice. The ligatures on the common duct are intact and the cut ends separated by about 1 cm. of scar tissue. The Eck fistula is of large size. The ligature on the portal vein above it obliterates the lumen and no collaterals enter above the ligature.

Microscopical Examination.—The kidneys show the usual type of suppurative nephritis associated with a good deal of hemorrhage. The omentum contains a large number of mononuclear phagocytes, filled with greenish yellow granular pigment. The pancreas is normal. The liver shows a definite central fatty degeneration involving about one third of each lobule. In these areas are seen scattered liver cells showing hyaline necrosis, but the majority of the liver cells show intact nuclei and contain fat droplets. There are large phagocytes containing yellow pigment in this same region. The liver cells elsewhere are normal. There is no increase in connective tissue. The injury done by the chloroform anesthesia in this case is obviously very slight.

ECK FISTULA. OBSTRUCTIVE JAUNDICE.

Dog C-67.—Small mongrel, female; weight, 12 pounds.

May 2. Ether anesthesia and Eck fistula produced as usual. The common bile duct is isolated and cut between the two ligatures. The urine obtained before operation showed no bile pigments.

May 3. Dog is in good condition. Urine contains bile pigments.

May 4. Bile pigments are more abundant in the urine.

May 5. Pigments are still more abundant and feces are clay colored. The skin and mucous membranes show a definite jaundice tinge.

May 6. Dog refuses food and is given milk by stomach tube. Bile pigments are very abundant in the urine.

May 6 to 16. Condition is somewhat improved and the animal eats fairly well. Bile pigments are positive in the urine. Skin and mucous membranes are definitely jaundiced.

May 20. Dog is in excellent condition, but there has been a little loss of weight. Conjunctivæ and mucous membranes are definitely jaundiced. Feces are clay colored. Urine contains a considerable amount of bile pigment.

May 26. Condition has remained uniform. 2 c.c. of urine contain bile pigment in demonstrable amount. 11 A. M. Washed red blood cells (40 c.c.), laked with an equal volume of water, were given intraperitoneally, as well as 300 c.c. of water by stomach tube. 2 P. M. Urine is deep red in color and contains a large amount of hemoglobin and the usual amount of bile pigment. 3 P. M. Urine is dark red in color. It contains a large amount of hemoglobin and apparently a little more bile pigment, 2 c.c. being used in all tests. 5 P. M. Very dark concentrated urine; bile pigments and hemoglobin are abundant.

May 27, 9 A. M. Urine collected during the night in the cage showed a large amount of bile pigment and considerable hemoglobin. The urine obtained by catheterization showed a definite increase in bile pigments above the previous day, but no hemoglobin.

May 28. Animal is in fair condition. Bile tingeing is marked in the mucous membranes and skin. Urine contains bile pigments in the usual amount. Hemoglobin is absent. Feces are clay colored. Weight, 8 pounds.

May 29. Animal found in convulsions. 11 A. M. Ether anesthesia and bled from carotid. The blood serum gives a strong test for bile pigment. Feces present in the large intestine are clay colored and give negative tests for stercobilin.

Autopsy.—Performed at once. All the body tissues, the subcutaneous tissues particularly, show definite bile tingeing. The thorax, heart, lungs, spleen, and kidneys are normal except for icterus. The bladder shows a very slight grade of cystitis. The peritoneum shows some brownish yellow staining, seen best in the omentum, due to the injection of red blood cells. There are a few adhesions about the site of operation. The gall bladder and common ducts are greatly dilated and quite tense. The common duct measures 1.5 cm. in diameter. It contains bright green bile. The liver is deep brown, rather translucent, and tough on section. The lobulation is even. The Eck fistula is wide open, and its edges are smooth. The ligature on the portal vein above the fistula completely occludes the lumen. Careful dissection shows a tiny venule running into the gastrohepatic omentum entering the portal vein above the ligature. The stomach is full of food. The intestine contains pasty, clay colored feces, and the mucosa is normal throughout.

Microscopical Examination.—The kidneys and spleen are normal. The liver lobules are of about normal size. There seems to be a little atrophy of the cell columns in the center of the lobules, and here the bile canaliculi are conspicuous and dilated with the usual yellowish green colloid. There is a slight increase in connective tissue about the portal spaces. Some of the lobules show a little fatty degeneration.

ECK FISTULA. EXTIRPATION OF THE SPLEEN. OBSTRUCTIVE JAUNDICE.

Dog C–55.—Black mongrel, male; weight, 27 pounds.

June 6. Ether anesthesia and Eck fistula established in the usual way. The spleen was extirpated at the same time and the common bile duct doubly ligated and cut between the ligatures. The urine obtained after the operation (10 c.c.) was negative for bile pigments.

June 7. Dog is in good condition. The urine is very dark and concentrated. It contains no hemoglobin but abundant bile pigments.

June 8 and 9. Bile pigments are present in the urine in large amounts. Urine collected from a clean metabolism cage was used in all the tests in this experiment and the bile pigments were constantly present in 5 c.c. of urine.

June 13. The skin and mucous membranes show a definite jaundice. Stools are clay colored. Tests for urobilin are negative. The bile pigments are abundant in the urine.

June 15 to 21. Animal is in good condition, and bile pigments are constantly present and easily demonstrated in 5 c.c. of urine.

June 24. Weight, 27½ pounds. Condition has remained the same. The mucous membranes and skin are definitely jaundiced.

June 29 to July 6. Condition remains the same. Dog eats vigorously and feces remain clay colored.

July 8. The jaundice seems to be less marked in the skin. The urine shows bile pigments.

July 10 to 19. Condition remains stationary. The skin is still yellowish in color, and bile pigments are constantly present in the urine. Weight, 26 pounds.

July 22. Urine is dark and concentrated and contains bile pigments. The stools are dry and clay colored. The tests for stercobilin are strongly positive.

July 23. Ether anesthesia and bleeding from the carotid. Blood serum (50 c.c.) gives a positive test for bile pigments. 70 gm. of omental fat give a positive test for bile pigments. 12 c.c. of urine taken from the bladder at autopsy give a strong test for bile pigments.

Autopsy.—Performed at once. The thorax, heart, lungs, pancreas, and kidneys are normal. The stomach and duodenum contain definitely bile-stained fluid and this material gives a positive test for bile pigments. The bile papilla and distal portion of the common duct contain definitely bile-stained, viscid material. The common duct has been cut across and the ends separated a distance of about 0.5 cm. The ligatures which closed the duct are found embedded in the wall. There is a definite fistula with a smooth lining which communicates between the upper and lower parts of the common bile duct, establishing communication and permitting a slow escape of bile under pressure into the duodenum. The common bile duct above the ligature is dilated to about 2 cm. in diameter, and its wall is thickened. The gall bladder also is dilated and thickened and contains dark, viscid bile.

The Eck fistula is of small size but remained wide open. The portal vein was not completely closed by the ligature above the fistula and there was a tiny pin point opening due to the fact that the intima had not been crushed by the ligature. There are no collaterals above the ligature. Obviously a small amount of portal blood gained entrance to the liver.

Microscopical Examinations.—The kidney is normal. The liver lobules appear normal, and there is a slight increase in connective tissue in the portal spaces, where one sees an accumulation of mononuclear wandering cells. The bile canaliculi, particularly in the centers of the lobules, are conspicuous and distended with a yellow colloid-like substance.

The five preceding experiments show that after an Eck fistula has been produced the ligation of the common bile duct will invari-

ably cause the appearance of bile pigments in the urine. A mild grade of icterus develops in the next few days and remains constant during the remainder of the experiment. The amount of bile pigments in the urine and the degree of icteric pigmentation is much less in Eck fistula dogs than in normal animals, but it is distinct and definite.

This observation does not agree with that of Voegtlin and Bernheim, who state that bile pigments are usually absent and that icterus never develops under such conditions. In the three experiments cited in their report, two of the dogs died from a large abscess developing in the hepatic region, and it is possible that this process may have modified the results. In their third experiment no statement is made concerning the condition of the gall bladder and ducts above the obstruction, and no tests were made for stercobilin to exclude the possibility of the duct having established its continuity in the way observed in two of the preceding experiments (dogs *C*–10 and *C*–55). They state that "the blood of the hepatic artery alone is sufficient for bile formation." What becomes of this pigment when the common duct is obstructed? Our experiments show that the bile pigments escape in the urine and stain the tissues as usual, although much less intensely than in the normal dog.

As the liver of an Eck fistula dog excretes less bile than a normal liver, it is to be expected that obstruction will cause less icterus and less bile pigment excretion in the urine. It seems obvious that the Eck fistula liver, being supplied with only one fourth of its usual blood volume, should secrete less bile and less bile pigments, and the observations bear this out. The icterus is less definite and the bile pigments are presumably less abundant in the tissues. But if the bile pigments are formed solely from broken down red blood cells, from hemoglobin, how is this to be explained? There is no evidence that in a normal animal more red blood cells are being destroyed than in an Eck fistula dog where the bile pigment excretion is so much diminished. When the liver is injured by chloroform, the icterus resulting therefrom varies in general directly with the amount of cell necrosis, but it follows in a dog with an Eck fistula as in a normal animal. We must explain this not by a capillary obstruction, but by a hemolysin (Joannovics) derived from the

injured liver cells. This hemolysin is as active in an Eck fistula dog as in a normal dog, indicating no increased resistance on the part of the red corpuscles of the Eck fistula dog.

In order to explain this lowered output of bile pigments by the Eck fistula liver it seems necessary to assume that the production of bile pigments may depend not wholly upon blood destruction, but may depend in part upon the activity of the liver epithelium. It is possible that the normal liver cells can form bile pigments under normal conditions out of substances not derived from hemoglobin. How else can we explain the great decrease in the formation of bile pigments in the Eck fistula liver where the red blood count and hematopoietic apparatus are normal and we are dealing with an atrophic liver supplied with only about one quarter of its normal blood volume?

There is additional evidence in favor of this view, as brought out by Sprunt working in this laboratory. He called attention to the fact that in hemochromatosis there is an enormous overproduction of iron-free and especially of iron-containing pigments with no involvement of the hematopoietic apparatus. The liver especially contains masses of this pigment, but there is no evidence of blood destruction and no anemia. One is forced to the conclusion that due to some perverted activity of the gland cells, there is a great overproduction of the pigments which must be formed from some other substance than hemoglobin. This supports the theory that the liver may be able to form bile pigments from substances other than hemoglobin.

SUMMARY.

Normal and Eck fistula dogs react in a similar manner to the intravenous injection of hemoglobin obtained from laked red cells of the same animal. Hemoglobin appears in the urine after a few minutes and bile pigments in one to one and one half hours. In this simple type of hematogenous jaundice the reaction is in no way influenced by shutting out the portal blood from the liver and cutting down its blood supply to about 25 per cent. of normal.

In a second type of hematogenous jaundice produced by chloroform anesthesia, which produces central liver necrosis, there is no essential difference between the normal and Eck fistula dog. The

Eck fistula dog, as a rule, is more resistant to this poison, but, given a definite liver necrosis, the jaundice developing will reach its maximum on the second day as in the normal animal. This jaundice must be explained in part by capillary biliary obstruction, but in part by a hemolysin formed in the injured liver cells (Joannovics and Pick).

Simple obstruction of the common duct when combined with an Eck fistula gives rise to a definite low grade icterus with bile pigment constantly present in the urine. Under these conditions after doubly ligating and cutting the common duct with separation of the cut ends, the lumen of the duct may be established and bile may enter the intestine by means of a fistulous tract between the cut ends of the bile duct.

The formation of bile and bile pigments is much less in an Eck fistula dog than in a normal animal and consequently the icterus is much less intense. This is probably due to a lessened activity of the liver cells because of decreased blood supply.

This observation does not harmonize with the current view that bile pigments are formed solely from hemoglobin, as there is no evidence of more hemolysis in a normal than in an Eck fistula dog.

This suggests that the bile pigment may be formed in part, at least, from other substances than hemoglobin, and, further, that bile pigment formation normally may depend in part upon the functional activity of the liver cell rather than upon the amount of hemoglobin supplied to it.

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ICTERUS.

A Rapid Change of Hemoglobin to Bile Pigment in the Circulation Outside the Liver.*

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The object of this communication is to submit evidence that hemoglobin can be transformed into bile pigment in the circulation when the liver has been excluded from participation in the reaction. It has been shown (Whipple and Hooper) that normal and Eck fistula dogs react essentially in a similar manner to an intravenous injection of hemoglobin, indicating that the liver is not the essential factor in this rapid change of hemoglobin to bile pigment which is completed within a few hours. Obviously the next step in this problem is the exclusion of the liver from any part in this chemical change. The earlier workers performed experiments with frogs (Löwit), pigeons (Stern), and geese and ducks (Mínkowski and Naunyn), but did not report successful work with mammals. Their conclusions were that jaundice and bile pigment formation could not develop without the liver (Stadelmann). Löwit claimed that in the frog bile pigments were produced without the agency of the liver, but other observers did not confirm his findings.

Widal and his co-workers have recently claimed that human cases present a form of icterus in which the bile pigment formation is hematogenous, or extrahepatic. They grant that bile pigment may be formed from hemoglobin which remains for a long time in the body tissues or serous cavities, but they state that in the group of cases under consideration there is a retention of bile pigment in the blood but not of bile salts and cholesterin. Stadelmann has shown that when hemoglobin was injected intravenously it increased the

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output of bile pigment, but decreased the output of bile acids. Widal argues that the absence of bradycardia speaks against the accumulation of bile salts in these cases, attributing the slowing of the heart-beat to the bile salts. It has been shown by King and Stewart that the bradycardia is due to the bile pigments, so Widal's observation indicates only the absence of a great amount of bile pigment in the blood, as compared with obstructive jaundice.

The cases show no disturbance of fat metabolism and on these grounds Widal claims that all the bile salts must gain admission to the intestine. It is not claimed by most workers that there is complete obstruction in these cases, but only a plugging of the canaliculi with dense tenacious bile and a partial capillary obstruction. The observations of Widal do not establish the point that some of the bile pigment production in these cases may be truly hematogenous and thus extrahepatic.

It might seem that removal of the liver would be the best way out of this difficulty, but there are many objections to this procedure. The operation is associated with much hemorrhage, laceration of the liver, and escape of fluids from the liver tissue (bile pigments, etc.), which can be rapidly absorbed from the body surfaces. This absorption of bile pigments has been mentioned in criticism of the experiments of Minkowski and Naunyn. The operation causes great surgical shock and lowers the blood pressure to a grave degree, which militates against an experiment of long duration. It is difficult to be sure that every bit of liver tissue is removed from the region of the vena cava and root of the diaphragm.

By means of the Eck fistula and ligation of both branches of the hepatic artery, almost all blood can be shut out of the liver and the animal dies in four to six hours from hepatic insufficiency. In this type of experiment a very small amount of blood can, of course, trickle into the liver by way of tiny collaterals in the suspensory ligament and liver capsule. It is possible, too, that there may be a certain amount of to and fro movement of blood in the larger hepatic veins associated with respiration. However, scarcely as much as 1 per cent. of the blood could come in contact with the perishing liver cells in this way. The experiments given below show that hemoglobin is changed to bile pigment in the circulation and

that the pigments appear in the urine in the normal interval. The question therefore arises as to whether the liver is the essential factor in the transformation.

METHOD.

The operative procedure is the same as that already described.[1] Huppert's test was used for the bile tests in the urine. In testing the plasma or serum the fluid was made definitely alkaline with the addition of sodium carbonate and calcium chloride. The mixture stood over night causing the calcium bile pigment compound to settle as a yellowish precipitate. After centrifugalization at high speed the supernatant fluid was poured off, and the precipitate was washed free from other coloring matter and collected again by centrifugalization. Finally the precipitate dissolved in acid alcohol gave the familiar blue green color when bile pigment was present. Fat and other tissues were first washed free from blood and then incubated at 40° C. in contact with two or three volumes of acid alcohol. After twelve to twenty-four hours the bile pigment when present appears in the alcohol, may be filtered off, concentrated, and tested in other ways.

ECK FISTULA. HEPATIC ARTERY LIGATED. HEMATOGENOUS JAUNDICE.

Dog C–95.—Large mongrel, female; weight, 36 pounds.

June 18. Dog is in excellent condition. 80 c.c. of urine give a negative test for bile pigments.

June 19, 9 A. M. Clear amber urine (50 c.c.) gives a negative test for bile pigments. Given 300 c.c. of water by stomach tube. 9.45 A. M. Ether anesthesia and bleeding from the jugular vein to obtain blood corpuscles for use later. Plasma (25 c.c.) gives a negative test for bile pigments. 10.30 A. M. Hepatic artery ligated. 10.55 A. M. Eck fistula is completed in the usual way and a ligature tied on the portal vein at the hilum of the liver. 11 A. M. 25 c.c. of red blood cells washed and laked with an equal volume of distilled water are given intravenously, followed by 400 c.c. of 0.9 per cent. salt solution. 11.13 A. M. Hemoglobin appears in the urine, and ether anesthesia is removed. Bile pigments first appeared in the urine at 12.30, when 60 c.c. gave a faintly positive test for bile pigments. Bile pigments and hemoglobin were constantly present until 4.20 P. M., when the dog is bled from the carotid. 25 c.c. of this plasma give a positive test for bile pigments. The plasma was a light claret color and showed oxyhemoglobin bands. Urine (14 c.c.) obtained from the bladder at autopsy gives a strong test for bile pigments.

Autopsy.—Performed at once. All the subcutaneous fat, which is abundant, shows obvious jaundice staining, the fat having a faint olive yellow tint, especially in the epicardium, perirenal, and omental fat. The heart and thymus are normal. The lungs contain a few small, purplish patches at the bases. The bronchi contain some vomitus. The spleen is engorged. The peritoneal cavity shows a few small clots around the site of operation and contains a little blood-stained fluid. The bladder is normal. The kidney shows definite chocolate staining of the cortex.

[1] Whipple, G. H., and Hooper, C. W., *Jour. Exper. Med.*, 1913, xvii, 593.

The liver is mottled, reddish gray in color, and rather soft. On section it is very juicy and is obviously undergoing autolytic digestion. The Eck fistula is perfect. The ligature on the portal vein obstructed the lumen completely, but one thread-like collateral opens into the portal vein above the ligature leading into the gastrohepatic omentum. Careful dissection showed that this collateral was included by the upper ligature on the hepatic artery so that no blood could come into the portal vein from this source. The ligatures on the hepatic artery closed this vessel completely, and occluded the greater number of the lymphatics running in the gastrohepatic omentum. This careful dissection shows that all arterial and portal blood was shut out of the liver, as far as the supply entering the hilum of the liver is concerned.

Dog C–95.

Time.	Urine in c.c.	Color.	Bile pigment test.	Pulse.	Respiration.	Temperature, C.	Remarks.
June 18	80	Amber	0				
June 19	50	Amber	0				300 c.c. of water by stomach tube.
9.55 to 10.55 A.M.	Eck fistula and hepatic artery ligated						
10.50 A.M.	7	Amber	0				Plasma (25 c.c.) negative for bile pigments.
11.00 A.M.	Laked red cells intravenously						400 c.c. of salt solution.
11.10 A.M.				180	120	37.0°	11.13 A.M. Oxyhemoglobin +
11.30 A.M.	16	Pink	0	180	90	37.3°	
12.00 M.	35	Claret	0	140	86	38.0°	
12.30 P.M.	62	Claret	+	120	80	39.0°	
1.00 P.M.	85	Claret	+	120	96	39.2°	Panting respirations.
1.30 P.M.	42	Claret	+	120	100	39.0°	200 c.c. of salt solution.
2.00 P.M.	68	Cherry	+	96	100	39.0°	Pulse irregular.
2.30 P.M.	50	Claret	++	140	100	39.0°	
3.00 P.M.	43	Pink	++	140	100	39.0°	200 c.c. of salt solution.
3.30 P.M.	46	Pink	++	130	—	39.0°	Muscular tremors.
4.00 P.M.	25	Cherry	+++	100	100	38.8°	100 c.c. of salt solution.
4.15 P.M.	—	——	—	52		38.8°	Urine flow scanty.
4.20 P.M.	Bled into oxalate						
Autopsy	14	Cherry	++	Plasma (25 c.c.) positive for bile pigment.			

Dog C–80.—Mongrel, female; weight, 28 pounds. This dog had been tested on a previous date (June 3) for its reaction to laked red blood cells injected intravenously.[2]

June 10. Dog is in perfect condition and urine is free from bile pigments.

June 13. Dog has been starved for twenty-four hours; weight, 27½ pounds. 9.40 A. M. Ether anesthesia and bleeding from the jugular vein. The red blood cells were washed and prepared for use later. 10 A. M. Ligation of the hepatic artery as usual. 10.40 A. M. Eck fistula is complete and the ligature is tied on the portal vein above the fistula. There was but little bleeding into the abdominal cavity. Urine is clear amber colored and negative for bile pig-

[2] Whipple, G. H., and Hooper, C. W., *loc. cit.*, p. 594.

ments. 25 c.c. of washed red blood cells laked with an equal volume of boiled distilled water are introduced into the external jugular vein together with 100 c.c. of 0.9 per cent. salt solution. Ether is then removed. 11.05 A. M. Hemoglobin appears in the urine and absorption bands of oxyhemoglobin are conspicuous on examination with the spectroscope. 11.40 A. M. Bile pigments are positive in the urine and hemoglobin is very abundant. 2 P. M. Muscular tremors and convulsions appear and the flow of urine ceases at once. 2.30 P. M. Dog is bled from the carotid and the blood pressure is quite low. Heart-beats are irregular in force and rhythm. Blood plasma and blood serum are cherry red in color and give positive tests for bile pigment.

Autopsy.—Performed at once. The abdominal cavity contains a little blood-stained fluid and no clots. The thymus is large. The heart is normal. The lungs show a few small, purplish areas in the bases. The spleen, liver, kidneys, and gastro-intestinal tract are normal. The Eck fistula is large and wide open. There are no clots on the edges. The ligature on the portal vein is just at the bifurcation in the hilum of the liver, above all collateral branches. The three ligatures on the hepatic artery are effective, and the ligature in the gastro-hepatic omentum above the arch of the hepatic artery includes most of the lymphatics draining the liver. The body fat everywhere, particularly in the intercostal spaces and over the heart, shows a delicate jaundice tinge, a faint olive yellow color.

Dog C–80.

Time.	Urine in c.c.	Color.	Bile pigment test.	Pulse.	Temperature, C.	Remarks.
June 10	10	Amber	o			
June 13						
9.40 A.M.		Eck fistula and hepatic artery ligated				Starved for 24 hours.
10.40 A.M.	—	Amber	o			Operation completed.
10.42 A.M.		Laked red cells given intravenously				100 c.c. of salt solution.
11.00 A.M.					37.7°	500 c.c. of salt solution.
11.05 A.M.	—	Pink	—	—		
11.40 A.M.	45	Cherry red	+	180	38.2°	Oxyhemoglobin+ 150 c.c. of salt solution.
12.10 P.M.	55	Cherry red	+	180	38.8°	
12.40 P.M.	70	Cherry red	++	200	39.3°	Condition good.
1.10 P.M.	33	Claret	+	200	——	100 c.c. of salt solution.
1.40 P.M.	22	Claret	++	240	39.2°	100 c.c. of salt solution.
2.10 P.M.	19	Claret	++	250	39.0°	100 c.c. of salt solution.
	Convulsions violent; artificial respiration					
2.30 P.M.	Death; bled from carotid into oxalate					Plasma (25 c.c.) and serum (25 c.c.) positive for bile pigment.

ECK FISTULA. HEPATIC ARTERY LIGATED. SPLEEN REMOVED. HEMATOGENOUS JAUNDICE.

Dog C–98.—Large mongrel, female; weight, 34 pounds.

June 24. Urine (25 c.c.) is negative for bile pigments.

June 25. Dog is active and strong; has been starved for twenty-four hours.

9.20 A. M. Urine (30 c.c.) gives a negative test for bile pigments. Ether anesthesia and bleeding from the jugular vein to obtain red blood cells for use below. Blood plasma (30 c.c.) gives a negative test for bile pigments. Laparotomy is performed and the hepatic artery ligated as usual. 10.30 A. M. Urine (55 c.c.) gives a negative test for bile pigment. Eck fistula is completed and the portal vein above the fistula ligated as usual. 10.40 A. M. 25 c.c. of red blood cells washed and laked with distilled water are introduced intravenously, followed by 200 c.c. of Ringer solution. During this time the spleen is extirpated and omental fat (15 gm.) removed. This gives a negative test for bile pigments and is of the usual appearance. 10.45 A. M. Ether discontinued. 10.55 A. M. Hemoglobin appears in the urine. Diuresis has not yet begun. 11 A. M. 100 c.c. of Ringer solution given intravenously. 11.10 A. M. Given 1/8 gr. of morphia intravenously. The urine first shows bile pigments at 1 P. M., after which time they are constantly present in the urine, together with oxyhemoglobin. Death took place at 3.30 P. M. after violent convulsions. Blood is obtained at once. Plasma (50 c.c.) gives a negative test for bile pigments.

Autopsy.—Performed at once. All the subcutaneous fat shows a definite jaundice tinge. The omental, pericardial, and subcutaneous, as well as the epicardial fat show a delicate olive yellow tint. 15 gm. of fat gave a strong test for bile pigments. The peritoneal cavity contains about 200 c.c. of blood-stained fluid together with a few clots. The viscera in general are normal.

The liver is definitely softened, mottled grayish red in color, soft and juicy on section, and is evidently undergoing autolysis and digestion.

Dog C–98.

Time.	Urine in c.c.	Color.	Bile pigment test.	Pulse.	Respiration.	Temperature, C.	Remarks.
June 24	25		0				Dog starved for 24 hrs.
June 25	30		0				
9.20 A.M.	Eck fistula and hepatic artery ligated						
10.30 A.M.	Spleen extirpated						Omental fat (15 gm.) negative for bile pigments.
10.30 A.M.	55	Amber	0	160	96	39.1°	
10.40 A.M.	Laked red cells intravenously						
10.55 A.M.		Pink					Oxyhemoglobin +
11.15 A.M.	15	Claret	0	140	24	39.3°	100 c.c. Ringer solution and 1/8 gr. morphia.
12.00 M.	80	Claret	0	132	28	39.3°	200 c.c. Ringer solution.
12.30 P.M.	55	Claret	0	140	56	39.3°	Condition excellent.
1.00 P.M.	50	Cherry	+(Faint)	100	26	38.9°	200 c.c. Ringer solution.
1.30 P.M.	60	Cherry	++	120	38	38.6°	
2.00 P.M.	54	Cherry	++	134	36	38.7°	
2.30 P.M.	48	Cherry	+++	120	48	39.0°	Muscular tremors.
3.00 P.M.	25	Claret	+++	160	100	39.0°	Muscular tremors increasing.
3.30 P.M.	13	Claret	+++	200	70	39.0°	Convulsions; death. Plasma (50 c.c.) negative for bile pigment; fat (15 gm.) positive for bile pigment.

The Eck fistula is perfect. There are no clots. The ligature on the portal vein above the fistula closed the vessel completely and no collaterals are found. The portal vein above the ligature shows a soft, flabby clot, indicating complete absence of circulation in this region. Ligatures on the hepatic artery are efficient and blood clots are found in this vessel as well. It is obvious that all blood entering the hilum of the liver had been shut out and that the liver was undergoing autolysis.

ECK FISTULA. HEPATIC ARTERY PARTIALLY LIGATED. HEMATOGENOUS JAUNDICE.

Dog C–83.—Mongrel, female; weight, 16 pounds.

May 28. Dog is tested with an intravenous injection of laked red cells.[3]

July 18. Weight, 14¾ pounds. Dog is in excellent condition and has been starved for twenty-four hours. 9.30 A. M. Urine contains no bile pigment. 10.35 A. M. Ether anesthesia. ⅛ gr. of morphia given subcutaneously. 10.40 A. M. Bled from carotid to obtain red blood cells used below. Plasma is water clear. 16 c.c. give a negative test for bile pigment. 11.10 A. M. Lapar-

Dog C–83.

Time.	Urine in c.c.	Color.	Bile pigment test.	Pulse.	Respiration.	Temperature, C.	Remarks.
July 18	10	Amber	0				Starved for 24 hrs.
11.40 A.M.	Eck fistula and hepatic artery ligated						
11.50 A.M.	15	Amber	0	140	30	36.0°	Ether discontinued.
11.55 A.M.	Laked red cells intravenously						200 c.c. salt solution.
12.40 P.M.	11	Claret	0	120	20	36.6°	12.23 P.M. Oxyhemoglobin +
1.10 P.M.	9	Cherry	0	80	20	37.4°	100 c.c. salt solution.
1.40 P.M.	10	Cherry	0	—	—	38.7°	
2.10 P.M.	12	Cherry	(?)	60	20	38.5°	Pulse irregular.
2.40 P.M.	11	Cherry	+(Faint)	72	20	39.6°	1/8 gr. morphia.
3.10 P.M.	9	Claret	++	60	20	39.4°	100 c.c. salt solution.
3.40 P.M.	13	Claret	+++	60	20	39.0°	
4.10 P.M.	24	Cherry	++	60	18	38.8°	
4.40 P.M.	27	Cherry	+	60	18	37.8°	Pulse irregular.
5.10 P.M.	23	Cherry	+	60	18	38.1°	
5.40 P.M.	20	Cherry	+	60	18	38.2°	
6.10 P.M.	17	Pink	+(Faint)	94	—	38.6°	Pulse irregular.
6.40 P.M.	20	Pink	+	94	—	39.0°	
7.10 P.M.	20	Pink	++	120	70	38.9°	Polypnea.
7.40 P.M.	21	Pink	+	96	30	38.5°	100 c.c. salt solution.
8.10 P.M.	23	Pink	0	96	30	38.3°	
8.40 P.M.	17	Pink	0	92	—	38.3°	
9.10 P.M.	16	Pink	+(Faint)	92	—	38.4°	
9.40 P.M.	16	Pink	+(Faint)	108	30	38.4°	
10.10 P.M.	10	Pale pink	0	88	30	38.5°	100 c.c. salt solution.
10.40 P.M.	20	Pale pink	0	120	30	38.6°	Dog drowsy.
11.10 P.M.	10	Pale pink	0	104	—	38.7°	Feces passed.
Autopsy	37	Brown	(?)				Omental fat (28 gm.) and cardiac fat (7 gm.) negative for bile pigments

[3] Whipple, G. H., and Hooper, C. W., *loc. cit.*, p. 595.

otomy and ligation of the hepatic artery in the usual way. 11.40 A. M. Eck fistula is completed as usual, and the portal vein above the fistula carefully ligated. 11.50 A. M. Ether anesthesia removed. Urine (15 c.c.) is negative for bile pigments. 11.55 A. M. 20 c.c. of red blood corpuscles washed with salt solution and laked with an equal volume of distilled water are given intravenously, followed by 200 c.c. of 0.9 per cent. salt solution. It is probable that death occurred between 12 and 1 o'clock during the night, judging from the amount of urine which collected in the bladder after the last observation at 11.10 P. M.

Autopsy.—July 19, 8 A. M. The heart contains blood clots. The lungs are normal. The spleen is enlarged and soft. The liver shows beginning post-mortem change. The peritoneal cavity contains about 75 c.c. of blood-tinged fluid which contained no clots. The Eck fistula is large. It shows one small clot adherent to the upper end of the fistula on the side toward the vena cava. The hepatic artery shows an anomalous branch given off close to the origin of the hepatic artery from the celiac axis, running in a thin fold of the gastro-hepatic omentum to the right portion of the right lobe. This branch was not included in the ligatures, and probably carried about one third of the volume of blood furnished by the arterial system to the liver. The blood supply was insufficient for the functional activity of the liver. It will be noted that the curve of appearance and excretion of bile pigments in the urine is practically identical with that of the normal dog. Omental fat (28 gm.) and cardiac fat (7 gm.) give negative tests for bile pigments.

ECK FISTULA. HEPATIC ARTERY LIGATED. HEMATOGENOUS JAUNDICE.

Dog C–85.—Fox terrier, female; weight, 15 pounds.

May 30. Urine negative for bile pigments. Ether anesthesia and Eck fistula established in the usual way.

June 1. Animal will not eat. Urine is clear amber and negative for bile pigments.

June 2, 9.30 A. M. Ether anesthesia and bleeding from the jugular vein to obtain the red blood cells used below. 10.45 A. M. Hepatic artery ligated as usual. There is considerable bleeding from the recent adhesions about the site of the previous operation. At the completion of the operation the red cells (27 c.c.), washed and laked with an equal volume of distilled water, are given intravenously, followed by 50 c.c. of normal salt solution. Urine (20 c.c.) is dark and concentrated and contains no bile pigments and no hemoglobin. Ether removed. 11.15 A. M. Hemoglobin appears in urine. 11.25 A. M. 200 c.c. of normal solution given intravenously. 11.30 A. M. Urine is deep red in color and contains abundant oxyhemoglobin. 40 c.c. are negative for bile pigments. 12 M. Urine cherry red in color. 80 c.c. give a positive test for bile pigments. 12.30 P. M. Urine is cherry red in color. 30 c.c. give a positive test for bile pigments. Muscular tremors are noted in the neck and legs. 12.55 P. M. Animal died.

Autopsy.—Performed at once. The organs in general are negative. The heart contains blood which clots slowly, requiring two to three hours for complete coagulation. There is an excess of antithrombin present. The peritoneal

cavity contains about 300 c.c. of blood-stained fluid. There are clots present and there has been a good deal of oozing from the adhesions broken at the time of the second operation. The Eck fistula is perfect and the ligature on the portal vein above the fistula closes the vessel completely and there are no collaterals above it. One of the ligatures on the arch of the hepatic artery was inefficient allowing a certain amount of blood coming from the collaterals through the pancreaticoduodenal branch to return and pass into the liver.

Dog C–85.

Time.	Urine in c.c.	Color.	Bile pigment test.	Remarks.
May 30	Eck fistula produced			
June 1	10	Amber	0	
June 2				
10.45 A.M.	Hepatic artery ligated			
10.50 A.M.	Laked red cells given intravenously			300 c.c. salt solution.
11.00 A.M.	20	Brown	0	Ether discontinued.
11.30 A.M.	40	Claret	0	11.15 A.M. Oxyhemoglobin + 200 c.c. salt solution.
12.00 M.	80	Cherry	+	
12.30 P.M.	30	Cherry	+	Muscular twitchings.
12.55 P.M.	Death			

Dog C–81.—Mongrel, female; weight, 16½ pounds.

May 30. Dog is in excellent condition. Ether anesthesia and Eck fistula produced in the usual manner.

May 31. Dog is in excellent condition. During the next month the animal was in fair condition except for a slight but steady loss in weight.

July 11. Weight, 13½ pounds. Dog is active and vigorous. 9.30 A. M. Urine is concentrated and dark brown. 17 c.c. give negative tests for bile pigments. 10.00 A. M. Ether anesthesia and bleeding to obtain red blood corpuscles to be used later. Blood plasma (30 c.c.) is negative for bile pigments. 10.40 A. M. Laparotomy and ligation of the hepatic artery in the usual way. 10.45 A. M. Red blood cells (15 c.c.) washed and laked with an equal volume of distilled water are introduced intravenously, followed by 270 c.c. of 0.9 per cent. salt solution. 10.55 A. M. Given ⅛ gr. of morphia and 1/300 gr. of atropin subcutaneously. Urine (22 c.c.) is dark, concentrated, and negative for bile pigments. 10.57 A. M. Hemoglobin appears in the urine. 11.20 A. M. Given 100 c.c. of 0.9 per cent. salt solution intravenously. 11.25 A. M. Urine is dark red in color. The oxyhemoglobin spectrum was characteristic. 12 c.c. give a negative test for bile pigments. 11.55 A. M. Urine is deep claret red in color. 17 c.c. give a faintly suspicious test for bile pigments. 12 M. Muscular tremors are noted in the neck and legs. 100 c.c. of 0.9 per cent. salt solution are given intravenously. 12.07 P. M. Injection of ⅛ gr. of morphia and 1/300 gr. of atropin intravenously. 12.20 P. M. Urine is deep claret red and contains a large amount of oxyhemoglobin. 10 c.c. give a faint but positive test for bile pigments. 12.20 P. M. The heart stops beating. The thorax is opened immediately and blood drawn from the heart with a pipette. The blood plasma is a deep claret red. 50 c.c. give a faint but positive test for bile pigments.

Autopsy.—Performed at once. The subcutaneous fat is scanty and of normal appearance. The heart shows numerous ecchymoses in the myocardium and subendocardial tissue. The spleen, pancreas, kidneys, and intestine are negative. The liver is pale yellow. Bile can be squeezed easily into the duodenum. The Eck fistula is perfect, but above the ligature on the portal vein are found two tiny collaterals just visible, leading from the gastrohepatic omentum into the portal vein at the hilum. The ligature on the portal vein occludes the lumen completely, but evidently a little collateral supply could gain entrance above the ligature. The hepatic artery was completely shut off from the liver.

Microscopical Examination.—The liver shows considerable atrophy particularly of the central parts of the lobules where the liver cells contain some pigment, and the blood vessels are dilated. There is practically no fat and only a small increase in connective tissue about the portal spaces. The myocardium shows no evidence of inflammation.

Dog C–81.

Time.	Urine in c.c.	Color.	Bile pigment test.	Pulse.	Respiration.	Temperature. C.	Remarks.
May 30	Eck fistula produced						
July 11							
8.30 A.M.	17	Brown	0				
10.40 A.M.	Hepatic artery ligated						Blood plasma negative for bile pigments.
10.45 A.M.	Laked red cells given intravenously						270 c.c. salt solution.
10.55 A.M.	22	Brown	0	—	—		1/8 gr. morphia; 1/300 gr. atropin.
11.15 A.M.				80	30	37.9°	10.57 A.M. Oxyhemoglobin in urine +
11.25 A.M.	12	Claret	0	—	—	——	100 c.c. salt solution.
11.55 A.M.	17	Claret	(?)	120	24	38.4°	Muscular tremors.
12.20 P.M.	10	Claret	+(Faint)	—	—	——	Death. Blood plasma (50 c.c.) faintly positive for bile pigment.

The six preceding experiments are uniform in many respects. After an Eck fistula and hepatic artery ligation with the introduction of laked red cells intravenously, bile pigments appear in the urine within two hours, and at times within one hour. This reaction is similar to that noted in the preceding contribution in normal dogs or in animals with a simple Eck fistula. Removal of the spleen does not modify this experiment. The dogs die within a varying period, the maximum being six hours; death is preceded by muscular tremors which become more intense and merge into violent tetanic convulsions. The pulse, respiration, and temperature show interesting variations. When the operation for Eck fistula is performed and the hepatic artery is ligated after an interval, the dogs do not

survive as long as when the two operations are done at once, but the results are the same. When the Eck fistula is performed several days or weeks previously there is an opportunity for the formation of collateral blood vessels in the adhesions around the operative area. In one experiment due to an anomaly of the hepatic artery, one branch was not secured. The dog lived for twelve hours, but reacted normally to hemoglobin injected intravenously and began to show clearing of the urine which contained only traces of the pigment during the last hours of life.

LIVER EXCLUDED. HEMATOGENOUS JAUNDICE INCREASING.

Dog C–89.—Large bull dog, female; weight, 23 pounds.

June 27, 10.30 A. M. Ether anesthesia and bleeding from the jugular vein. The plasma after repeated centrifugalization was clear, but of a faint, pinkish tinge, and showed oxyhemoglobin bands when examined by the spectroscope. The leucocytic layer was several times thicker than normally. Urine (22 c.c.) is highly colored and concentrated, and gives a positive test for bile pigment. 11.05 A. M. Hepatic artery ligated as usual. 11.35 A. M. The Eck fistula is completed and the ligature tied on the portal vein. Omental fat (10 gm.) is negative for bile pigments. 11.40 A. M. Ether discontinued. 11.45 A. M. 250 c.c. of Ringer solution is introduced intravenously, and shortly afterwards ⅛ gr. of morphia dissolved in 50 c.c. of Ringer solution. 6 P. M. Animal is bled from the carotid into the oxalate. The plasma is a pale clear red and oxyhemoglobin bands are conspicuous. Plasma (50 c.c.) gives a positive test for bile pigments.

Autopsy.—Performed at once. The heart shows tiny vegetations on the mitral valve associated with hemorrhages 1 or 2 mm. in diameter on the valves above these vegetations and over the tips of the papillary muscles. The lungs are normal. The peritoneal cavity contains 30 c.c. of blood-tinged fluid. The spleen shows a few very small infarcts below the capsule and is somewhat swollen. The liver shows a mottled, reddish gray color. It is definitely softened and undergoing autolysis. The gall bladder and duodenum contain bile, and there is no obstruction in the course of the common bile duct. Ligatures on the hepatic artery shut off the arterial blood completely. The Eck fistula is large and patent. There were two small clots on the edge toward the vena cava. The ligature on the portal vein above the fistula closed the vessel completely and was placed just at the bifurcation of the vein at the hilum of the liver. No collaterals entered the portal vessels above this point. The kidneys are negative.

This experiment shows a cause for jaundice which may be present in dogs apparently in good health. The animal had a recent verrucose mitral endocarditis with a leucocytosis and definite hemolysis, the plasma showing definite bands of oxyhemoglobin and a faint

Dog C–89.

Time.	Urine in c.c.	Color.	Bile pigment test.	Pulse.	Respiration.	Temperature, C.	Remarks.
June 26	20		0				
June 27							
10.30 A.M.	Eck fistula and hepatic artery ligated						
10.45 A.M.	22	Brown	+				
11.33 A.M.	Operation completed						Omental fat (10 gm.) negative for bile pigments.
11.40 A.M.	3	Brown	(?)	200	60	36.8°	300 c.c. Ringer solution and 1/8 gr. morphia. Ether discontinued.
12.30 P.M.	38	Amber	0	160	30	37.5°	
1.00 P.M.	24	Amber	+(Faint)	200	96	37.4°	
1.30 P.M.	27	Amber	+	140	88	38.0°	200 c.c. Ringer solution.
2.00 P.M.	64	Amber	+	120	32	37.8°	
2.30 P.M.	45	Amber	++	120	48	38.0°	
3.00 P.M.	52	Amber	+	160	42	38.1°	
3.30 P.M.	26	Amber	+	160	38	38.0°	
4.00 P.M.	21	Amber	+	180	40	37.9°	100 c.c. Ringer solution.
4.30 P.M.	37	Amber	++	140	32	37.6°	
5.00 P.M.	16	Amber	++	140	24	37.4°	150 c.c. Ringer solution.
5.30 P.M.	50	Amber	+	120	28	37.3°	Muscular tremors.
6.00 P.M.	16	Amber	+				
6.02 P.M.	Bled from carotid						Plasma (50 c.c.) pale claret colored. Strongly positive for oxyhemoglobin and bile pigment.

pink color. It has been recognized that spontaneous jaundice might occur in dogs, and in the spring it is not uncommon, especially associated with a mild grade of distemper. This dog after the Eck fistula and hepatic artery ligation showed an increase in bile pigments in the urine, body fluids, and tissues. It is probable that there was increased hemolysis and absorption of hemoglobin from the peritoneum around the area of operation, as the plasma at autopsy was of a deeper pink than at the beginning of the experiment.

ECK FISTULA. HEPATIC ARTERY LIGATED. CONTROL.

Dog C–41.—Mongrel terrier, female; weight, 18 pounds.

July 16. Dog is in excellent condition. Urine (20 c.c.) is amber clear and contains no bile pigment.

July 17. Dog has been starved for twenty-four hours. 8.15 A. M. Urine (15 c.c.) contains no bile pigments. 10.45 A. M. Ether anesthesia. Bleeding from the jugular vein into the oxalate. The plasma after centrifugalization was water clear with a faint amber tinge. 24 c.c. give a negative test for bile pigments. This clear plasma gives no oxyhemoglobin bands when examined with

the spectroscope. The dog is given ⅛ gr. of morphia and 1/300 gr. of atropin subcutaneously.

The operation was done with more difficulty than usual and a small Eck fistula was made. There was considerable bleeding into the peritoneal cavity at this time, but it was readily checked and most of the blood mopped out. The hepatic artery was ligated in three places as usual after the Eck fistula had been completed and finally the ligature on the portal vein above the fistula was tied. This gave an opportunity for beginning thrombus formation on the edges of the fresh fistula and the color of the intestine was rather bluish, indicating considerable obstruction to the venous flow.

11.45 A. M. 280 c.c. of 0.9 per cent. salt solution given intravenously. 11.55 A. M. Urine (30 c.c.) is rather turbid, amber yellow, and bile pigments are negative. Ether anesthesia discontinued. 12 M. 100 c.c. of 0.9 per cent. salt solution are given intravenously. Diuresis is not active. 12.30 P. M. 100 c.c. of 0.9 per cent. salt solution given intravenously. 12.45 P. M. Urine (5 c.c.) is concentrated and turbid, and negative for bile pigments. 4.45 P. M. Animal dies in severe convulsion. The heart beats vigorously for one minute after the respirations ceased. The blood is removed immediately after death with a clean pipette from the auricle. Plasma after centrifugalization shows a pale pink tinge, and the spectroscope shows oxyhemoglobin bands. 50 c.c. of plasma give a negative test for bile pigments.

Autopsy.—Performed at once. The peritoneal cavity contains unclotted blood, which is to be explained undoubtedly by the violent convulsion just before

Dog C–41.

Time.	Urine in c.c.	Color.	Bile pigment test.	Pulse.	Respiration.	Temperature, C.	Remarks.
July 16	20	Amber	0				Dog starved for 24 hrs.
July 17	15	Brown	0				
10.45 to 11.40 A.M.	Eck fistula and hepatic artery ligated						
11.45 A.M.	Salt solution intravenously, 280 c.c.						
11.55 A.M.	30	Amber	0	140	64	37.4°	100 c.c. salt solution. Ether discontinued.
12.30 P.M.				140	18	37.3°	100 c.c. salt solution. Diuresis poor.
12.45 P.M.	5	Brown	0	—	—	——	100 c.c. salt solution.
1.15 P.M.	8	Brown	0	—	—	——	
1.45 P.M.	14	Amber	0	124	18	37.7°	
2.15 P.M.	12	Amber	0	128	20	38.0°	
2.45 P.M.	20	Amber	+(Faint)	98	24	38.1°	100 c.c. salt solution.
3.15 P.M.	42	Amber	0	56	20	38.2°	Muscular tremors.
3.45 P.M.	57	Pale amber	(?)	120	26	38.1°	100 c.c. salt solution. 1/4 gr. morphia; 1/150 gr. atropin.
4.15 P.M.	67	Pale	0	160	16	38.0°	
4.30 P.M.	—	——		160	20	——	100 c.c. salt solution.
4.45 P.M.	Death; convulsions						
Autopsy	23	Amber	(?)				Plasma (50 c.c.) and peritoneal fluid (40 c.c.) pale pink in color and negative for bile pigment.

death. There are also a few blood clots to be seen. The thorax, heart, and lungs are normal. The intestine shows a moderate congestion of the serous surface, but a deep red velvety mucosa indicating considerable obstruction to the portal outflow. The Eck fistula shows partial occlusion by a fresh thrombus adherent to one margin. The ligature on the portal vein above the Eck fistula completely occluded the lumen and careful dissection revealed no collaterals above it opening from the gastrohepatic omentum into the portal vein. The subcutaneous, perirenal, and mediastinal fat are all normal.

Dog C–105.—Mongrel hound, female; weight, 24 pounds.

July 12. Urine is concentrated. 25 c.c. are negative for bile pigments.

July 13. Dog has been starved for thirty-six hours. 8.30 A. M. 25 c.c. of concentrated urine are negative for bile pigments. 9.45 A. M. Ether anesthesia with bleeding from the jugular vein. Plasma is water clear, and the spectrum is negative for oxyhemoglobin. 25 c.c. of plasma give a negative test for bile pigments. 11.06 A. M. The Eck fistula is completed with much bleeding and injury to the tissues because of a band which had been previously placed upon the left renal vein. This old operation had been associated with much inflammatory reaction and thickening of the inferior vena cava. There was a slight amount of laceration of the liver and about 30 c.c. of blood escaped into the peritoneal cavity. This was mopped out carefully. The fistula was of small size. 11.10 A. M. The hepatic artery is ligated securely as usual. 11.15 A. M. 250 c.c. of 0.9 per cent. salt solution are introduced into the jugular vein. 1/8 gr. of morphia and 1/300 gr. of atropin are given subcutaneously. 11.25 A. M. 200 c.c. of salt with 1/100 gr. of hirudin are given intravenously, the hirudin to prevent any thrombosis in the neighborhood of the Eck fistula. 4.30 P. M.

Dog C–105.

Time.	Urine in c.c.	Color.	Bile pigment test.	Pulse.	Respiration.	Temperature, C.	Remarks.
July 12	25	Brown	0				Dog starved for 36 hrs.
July 13	25	Brown	0				
9.45 to 11.10 A.M.	Eck fistula and hepatic artery ligated Plasma—Oxyhemoglobin 0—Bile pigments—0						
11.15 to 11.30 A.M.	450 c.c. salt solution given intravenously						1/8 gr. morphia, 1/300 gr. atropin.
11.40 A.M.	4	Brown	0	188	42	39.0°	
12.10 P.M.	4	Brown	0	160	40	38.9°	
12.40 P.M.	10	Amber	0	160	40	39.0°	
1.10 P.M.	15	Brown	0	200	36	38.6°	100 c.c. salt solution.
1.40 P.M.	22	Brown	(?)	160	36	39.4°	
2.10 P.M.	25	Brown	(?)	160	40	39.3°	100 c.c. salt solution.
2.40 P.M.	18	Brown	?)	154	160	39.7°	Panting respiration.
3.10 P.M.	17	Brown	+(Faint)	160	70	39.5°	Muscular tremors.
3.40 P.M.	23	Red	+	200	80	39.4°	
4.10 P.M.	18	Brown	+(Faint)	160	28	39.0°	100 c.c. salt solution.
4.30 P.M.	24	Brown	(?)	—	—	——	Death. Plasma (50 c.c.) delicate pink; negative for bile pigment. Peritoneal fluid (20 c.c.) negative for bile pigment.

Death in convulsions. The thorax is opened immediately and blood taken from the heart with a clean pipette. Plasma obtained after repeated centrifugalization is a definite pink color showing conspicuous oxyhemoglobin bands. 50 c.c. give a negative test for bile pigments.

Autopsy.—Performed at once. The peritoneal cavity contains about 50 c.c. of claret colored fluid which clots in three or four minutes. The serum squeezed out is of a cherry red color. 20 c.c. give a negative test for bile pigments. The heart, lungs, intestine, and spleen are all normal. The liver shows a mottled reddish gray color, and is soft and juicy. On section the liver is evidently undergoing autolysis. The gall bladder is flabby and collapsed. The common duct is normal. The Eck fistula is perfect. One small clot was found on the edge but the lumen is clear. The ligature tightly occludes the portal vein above the fistula and no collaterals open in above the ligature. The portal vein above the ligature shows a definite clot adherent to the intima injured by clamping and tying the vessel. The hepatic artery is completely occluded by ligatures.

ECK FISTULA. CONTROL.

Dog C-99.—Fox terrier, female; weight, 14 pounds.

July 3, 9.30 A. M. Dog is in good condition. 60 c.c. of concentrated urine give a negative test for bile pigments. 11.15 A. M. Ether anesthesia. Operation and production of Eck fistula in the usual way. There was some bleeding (20 c.c.) at the time of operation, but this was controlled and the abdomen mopped out. The abdominal cavity contains a little blood when closed. 12.15 P. M. The fistula is complete and the ligature tied on the portal vein above the fistula. 12.40 P. M. Given 200 c.c. of Ringer solution in which was dissolved ⅛ gr. of morphia. Bile pigments in small amounts appear in the urine after two hours, but disappear before the end of the observation. 3 P. M. Dog is bled from the jugular vein into the oxalate in the usual way. The plasma shows a

Dog C-99.

Time.	Urine in c.c.	Color.	Bile pigment tests.	Pulse.	Respiration.	Temperature, C.	Remarks.
July 3	60	Brown	0	120	40	38.1°	
12.15 P.M.	Eck fistula completed						Ether discontinued. 200 c.c. Ringer solution and 1/8 gr. morphia.
12.40 P.M.	4	Brown	0	160	120	38.3°	
1.30 P.M.	5	Brown	0	160	180	38.3°	
2.00 P.M.	7	Brown	+(Faint)	160	30	37.9°	200 c.c. Ringer solution.
2.30 P.M.	15	Amber	+	—	—	—	
3.00 P.M.	22	Amber	+	140	28	37.8°	Blood plasma and oxyhemoglobin +.
3.30 P.M.	18	Amber	(?) +	140	24	37.6°	
4.00 P.M.	25	Amber	(?) +	140	24	37.4°	
4.30 P.M.	15	Amber	(?)	160	26	37.5°	
5.00 P.M.	13	Amber	0	140	28	37.6°	
July 4	4	Turbid brown	0	—	—	—	
July 19	Autopsy						

definite pink tinge and the spectrum shows the presence of oxyhemoglobin bands. This plasma was obtained after two separate centrifugalizations with pipetting off of the supernatant fluid, and indicates that there must be more or less absorption of hemoglobin from the site of operation.

July 4. Urine is negative for bile pigment.

July 19. Ether anesthesia and bleeding from the carotid. The plasma and fat obtained at autopsy give negative tests for bile pigments. The heart contains many filariæ. The lungs, spleen, and intestine are normal. The liver is somewhat pigmented and pale brown in color. The Eck fistula is perfect. The ligature present at the hilum of the liver completely occludes the portal vein and there are no collaterals opening in above this ligature.

The three preceding experiments serve as controls on the operative procedure. Bile pigments in small amounts may appear in the urine following the operation, but the appearance of the pigments is late and the duration short. Blood examination shows the presence of a definite amount of hemoglobin in the plasma from which the bile pigments in very small amounts are formed. The presence of this hemoglobin in the plasma is probably due to absorption from the field of operation, especially the peritoneal cavity, but may be due in part to the long anesthesia and the introduction of large amounts of normal saline or Ringer solution to promote a brisk diuresis. The fluid at autopsy obtained from the peritoneal cavity gives a negative test for bile pigments, so it is unlikely that any preformed bile pigments were absorbed from this region following the operative manipulations.

LIVER AND ABDOMINAL VISCERA EXCLUDED. HEMATOGENOUS JAUNDICE.

Dog C–97.—Strong brindle bull, female; weight, 32 pounds.

July 16. Dog is in excellent condition. 20 c.c. of urine give a negative test for bile pigments.

July 17, 8.30 A. M. 20 c.c. of urine give a negative test for bile pigments. 10.45 A. M. Given ⅛ gr. of morphia subcutaneously. 11 A. M. Ether anesthesia with bleeding from the external jugular vein. The plasma is slightly turbid due to lipemia, but the spectrum is negative. Bile pigment test (25 c.c.) is negative. 11.30 A. M. Artificial respiration is established and the mammary vessels ligated through the thorax. 11.40 A. M. The thoracic aorta just below the subclavian artery is closed securely by a screw clamp. 11.45 A. M. The abdominal aorta is clamped at the celiac axis and the inferior mesenteric artery is clamped securely. The aorta is clamped again just above its bifurcation. The portal vein at the hilum of the liver is securely ligated. The vena cava is left intact. 11.50 A. M. After these vessels are secured, 25 c.c. of washed laked red blood cells are introduced intravenously. The animal is kept under

light anesthesia during the entire observation. 12.20 P. M. The color of the tongue and mucous membrane is a bright vivid pink, and pressure shows that the capillary circulation is excellent. The heart pulsation is strong and regular. 12.45 P. M. Many of the smaller vessels which were severed at the time of operation through the thorax begin to bleed, evidently due to rise in blood pressure. They are carefully secured by clamps. 1 P. M. Dog is in excellent condition and was given 50 c.c. of 0.9 per cent. salt solution containing 0.1 per cent. dextrose. 1.40 P. M. The color of the tongue and mucous membranes is excellent. 2.15 P. M. Muscular tremors are noted in the neck and forelegs. The heart pulsations are strong and regular. Given 50 c.c. of 0.9 per cent. salt solution containing 0.1 per cent. dextrose and ⅛ gr. of morphia. 2.25 P. M. Muscular tremors are becoming more violent. The dog is given ⅛ gr. of morphia subcutaneously in the neck. 3.15 P. M. The color of the tongue and mucous membranes is good and the circulation is active. Muscular tremors have ceased. 3.30 P. M. Given 50 c.c. of 0.9 per cent. salt solution with 0.1 per cent. dextrose. 4 P. M. Given 50 c.c. of a similar solution intravenously. 4.15 P. M. The color of the mucous membranes is bright pink. 4.40 P. M. The heart stops beating. There are no tetanic convulsions. The thorax is opened at once and blood is drawn from the heart with a clean pipette. The blood plasma after centrifugalization is pale claret red. The spectrum shows conspicuous oxyhemoglobin bands. 50 c.c. of plasma give a strong positive test for bile pigments. 25 c.c. of serum likewise give a strong test for bile pigments.

Autopsy.—Performed at once. The subcutaneous, intercostal, supraclavicular, mediastinal, and epicardial fat is of a canary yellow color suggesting a definite jaundice, and gives a positive test for bile pigments. 6 gm. of epicardial fat give a strong positive bile pigment test; 8 gm. of intercostal fat give a fainter positive test; 13 gm. of mediastinal fat give a faintly positive test; 27 gm. of supraclavicular and subcutaneous fat give a strong positive test for bile pigment. Fat from the omentum and about the kidneys is normal in appearance. 18 gm. of this fat give negative tests for bile pigments. The heart is dilated and shows a few ecchymoses below the intima of the left ventricle. The lungs and viscera in general are normal. The intestine, stomach, and spleen show definite post-mortem changes, with much gas formation in the lumen of the small intestine. The liver is small and contains less blood than

Dog C–97.

	Bile pigment tests.		
	Urine.	Blood.	Fat.
Before operation	20 c.c. negative	25 c.c. negative	
	Liver and abdominal viscera excluded for 5 hours		
After operation		Plasma (50 c.c.) ++	Epicardial (6 gm.) ++
		Serum (25 c.c.) ++	Intercostal (8 gm.) +
			Mediastinal (13 gm.) +
			Supraclavicular (27 gm.) ++
			Omental (18 gm.)—
			Perirenal (18 gm.)—

normally and shows no gross evidence of autolysis. The gall bladder is flaccid and the common duct is patent. The portal vein shows a trifling amount of congestion but no marked engorgement, indicating the absence of any collateral supply to this area. The peritoneal cavity contains about 5 c.c. of blood-tinged fluid.

The preceding experiment answers most of the objections that can be brought forward in criticism of the Eck fistula hepatic artery ligation group of experiments. In this experiment the portal and arterial blood is shut out from the liver and abdominal viscera in general, and with a clamp upon the aorta below the subclavian artery there can be no active circulation through any of the diaphragmatic and ligamentary collaterals to the liver. This practically excludes all seepage through the capsular collaterals of the liver and means that the liver is cut off from all active association with the circulating blood. Because of the respiratory movement, a to and fro motion of the blood in the hepatic veins and vena cava may take place and bring a certain amount of blood in contact with liver tissue. That this could be sufficient for any active participation of the liver cells in the transformation of hemoglobin into bile pigments is unlikely. The series of experiments given below answers this question. The vena cava was left intact so that it might return the small amount of blood which escapes by collaterals along the costal margin, in the vertebral column, and attachments of the diaphragm.

HEAD AND THORAX CIRCULATION. HEMATOGENOUS JAUNDICE.

Dog C–106.—Large bull dog, female; weight, 38 pounds.

July 15. Dog is in excellent condition; starved for twenty-four hours. 9 A. M. 25 c.c. of concentrated urine are negative for bile pigments. 10.20 A. M. Given ⅛ gr. of morphia and 1/300 gr. of atropin subcutaneously. 10.30 A. M. Ether anesthesia and bleeding from the external jugular vein. The plasma is water clear. 35 c.c. of plasma give negative tests for bile pigments. 10.35 A. M. Given ⅛ gr. of morphia and 1/300 gr. of atropin subcutaneously. 11 A. M. Artificial respiration begun. Mammary vessels ligated. 11.05 A. M. The thoracic aorta is clamped just below the subclavian artery. 11.10 A. M. The abdominal aorta is ligated at the celiac axis. Wire ligatures are placed through the recti muscles and along the costal margin to control the collateral vessels. 11.15 A. M. The vena cava is ligated above the diaphragm and 25 c.c. of laked corpuscles are introduced intravenously. 11.30 A. M. The color of the tongue and mucous membranes is good. The heart pulsation is strong and regular. 11.45 A. M. 50 c.c. of normal salt solution containing 0.1

per cent. dextrose are given intravenously. 12.25 P. M. The condition is uniformly excellent. 25 c.c. of salt solution are given intravenously as before. 1 P. M. The color of the tongue is excellent. The heart pulsates regularly. 25 c.c. of 0.9 per cent. salt solution given intravenously. 1.15 P. M. Muscular tremors are noted in the neck. 1.30 P. M. The heart stops pulsating but the circulation had been vigorous and active up to within five minutes before this time. Blood taken at once from the heart clotted in the normal way and the serum is a cherry red color. 50 c.c. of this serum give a strong test for bile pigments.

Autopsy.—Performed at once. The fat of the thorax, mediastinum, and epicardium shows a definite jaundice tinge as compared with that below the diaphragm. The supraclavicular fat (27 gm.) and mediastinal fat (16 gm.) give positive tests for bile pigments; the epicardial fat (12 gm.) gives a strong positive test for bile pigments; the omental fat and the subcutaneous fat below the diaphragm are normal in color. The omental fat (22 gm.) gives a negative test for bile pigments, the inguinal fat (30 gm.), and retroperitoneal fat (38 gm.) likewise give negative tests. The heart is greatly dilated. The lungs are normal. The spleen is considerably engorged. The liver is distended with blood. The vena cava and branches together with the kidneys are also engorged. All the ligatures at the places indicated are effective and close the lumen of the vessels completely.

Dog C–106.

	Bile pigment tests.		
	Urine.	Blood.	Fat.
Before operation	25 c.c. negative	35 c.c. negative	
	Head and thorax circulation, 2¼ hrs		
After operation		Serum (50 c.c.) +++	Supraclavicular (27 gm.) +
			Mediastinal (16 gm.) +
			Epicardial (12 gm.) ++
			Omental (22 gm.) −
			Inguinal (30 gm.) −
			Retroperitoneal (38 gm.) −

Dog C–13.—Large pointer, male; weight, 44 pounds.

June 22. Dog is in excellent condition; starved for twenty-four hours. 9.30 A. M. Ether anesthesia with bleeding from the femoral artery. Plasma is water clear. 25 c.c. give a negative test for bile pigments. 10 A. M. Given ¼ gr. of morphia dissolved in 50 c.c. of 0.9 per cent. salt, intravenously. Artificial respiration begun. 10.15 A. M. The aorta ligated with wire just beyond the subclavian artery. 10.20 A. M. The mammary vessels are ligatured and wire ligatures placed across the recti muscles and along the costal margin to control the collateral circulation. 10.30 A. M. The vena cava is ligated above the diaphragm and immediately afterwards 25 c.c. of red blood cells laked with distilled water are given into the jugular vein, followed by 85 c.c. of 0.9 per cent. salt solution. 11.10 A. M. 50 c.c. of salt solution is given into the jugular vein. 12.15 P. M. The pulse is strong and regular. The tongue is bright red. 25 c.c. of salt solution are given intravenously. 12.50 P. M. The color of the

tongue has become bluish. 1 P. M. The heart stopped beating. The thorax is opened and blood is taken from the heart with a clean pipette. Plasma after centrifugalization is of a deep claret red. 30 c.c. of serum give a positive test for bile pigments. 50 c.c. of serum in the second test give a strong positive test for bile pigment.

Autopsy.—Performed at once. The fat over the thorax in the intercostal spaces, in the supraclavicular fossa, in the mediastinum, and the epicardium shows a definite jaundice tinge. 4 gm. of this material extracted with hot acid alcohol give a pale green color indicating the presence of bile pigment. The fat in the omentum and abdomen is normal in appearance. The line of demarcation between the normal and abnormal fat is along the costal margin, one fading gradually into the other. 4 gm. of omental fat treated in a similar way give no color reaction test. The heart and lungs are negative. The spleen is engorged, purple, and very soft. The liver is acutely engorged and deep purple. The ligature on the vena cava occludes the lumen completely and there are soft purple clots in the vein below the ligature extending into the hepatic veins. The intestinal mucosa shows a good deal of engorgement, giving it a pinkish color. The kidneys are a deep purple color and show great vascular engorgement. The ligature on the aorta had cut into the wall with considerable injury to the vessel, and it is possible that a little blood could pass the ligature.

Dog C–102.—Bull dog, female; weight, 27 pounds.

July 9, 9 A. M. 20 c.c. of urine give a negative test for bile pigments. 10.15 A. M. Dog is given 600 c.c. of water by stomach tube. 11.10 A. M. Ether anesthesia and bleeding from the jugular vein. Plasma is water clear. 50 c.c. give a negative test for bile pigment. 11.20 A. M. Given ⅛ gr. of morphia dissolved in 25 c.c. of Locke's solution, intravenously. 11.35 A. M. Artificial respiration started. 11.40 A. M. Ligation of mammary vessels. 11.46 A. M. The aorta is ligated with wire just below the subclavian artery. 11.50 A. M. A clamp is put across the aorta including the celiac axis. Heavy wire ligatures are put across the recti muscles and along the costal margin to occlude collaterals. 12 M. The vena cava inferior is ligated just above the diaphragm and 25 c.c. of washed, laked red blood cells are introduced intravenously with 50 c.c. of Locke's solution. 1 P. M. Condition is very good. The tongue and mucous membranes are a bright pink color. The heart-beat is strong and regular. Given 50 c.c. of Locke's solution intravenously. 1.45 P. M. Muscular tremors are noted in the neck muscles. 1.55 P. M. The heart stops beating. The thorax is opened at once and blood taken from the heart. The plasma after centrifugalization appears claret red. Oxyhemoglobin bands are conspicuous. 65 c.c. of plasma give a very faint but positive test for bile pigments.

Autopsy.—Performed at once. Fat in the supraclavicular, intercostal, and epicardial tissue has a definite canary yellow color as compared with the fat in the groin and omentum. 20 gm. of supraclavicular fat give a positive test for bile pigments; 5 gm. of epicardial fat give a positive suspicious test for bile pigments; 25 gm. of fat from the groin give a negative test for bile pigments. Examination of the vessels shows that all the ligatures are effective. The abdominal viscera as usual show engorgement.

HEAD AND THORAX CIRCULATION. JAUNDICE NOT POSITIVE.

Dog C–100.—Large male; weight, 36 pounds.

July 5. Dog has been starved for twenty-four hours and is in excellent condition. 9 A. M. Given 800 c.c. of water by stomach tube. 10.20 A. M. Ether anesthesia. A large amount of urine is collected while the animal is going under the anesthetic. 50 c.c. of this urine are negative for bile pigments. 10.25 A. M. Dog given intravenously ¼ gr. of morphia dissolved in a little salt solution, after bleeding from the jugular vein. Plasma after centrifugalization is water clear. 25 c.c. give a negative test for bile pigment, and the spectrum shows no oxyhemoglobin bands. 10.40 A. M. Artificial respiration begun. 11 A. M. The aorta occluded by a strong clamp is placed just below the subclavian artery and the mammary vessels are ligated. 11.10 A. M. The vena cava is tied above the diaphragm. 11.15 A. M. Red blood cells (25 c.c.) laked with an equal volume of distilled water are given intravenously, followed by 50 c.c. of Ringer solution. 11.45 A. M. The color of the mucous membranes and tongue is bright pink. The heart-beat is regular and strong. 12.15 P. M. Condition continues the same. 1.15 P. M. Condition is excellent. 1.45 P. M. Muscular tremors are noted in the neck and the color of the tongue is poor. 1.50 P. M. Death. The thorax is opened and blood is taken directly from the heart. After centrifugalization the plasma is claret red in color. The tests made with 25 c.c. of plasma were not positive but give a suggestive reaction for bile pigments.

Autopsy.—Performed at once. The fat in the abdominal cavity is normal in appearance, except for congestion. The fat in the supraclavicular fossa has a faint suggestion of bile tingeing, but it was not definite. The epicardial fat also

BILE PIGMENT TESTS. DOG C–13.

	Urine.	Blood.	Fat.
Before operation		25 c.c. –	———
After operation	Head and thorax circulation for 2½ hrs.		
		Serum (30 c.c.) +	Epicardial (4 gm.) +
		Serum (50 c.c.) ++	Omental (4 gm.) –
		DOG C–102	
Before operation	20 c.c. –	50 c.c. –	———
After operation	Head and thorax circulation for 2 hrs.		
		65 c.c. + (faint)	Supraclavicular (20 gm.) +
			Epicardial (5 gm.) +(?)
			Inguinal (25 gm.) –
		DOG C–100	
Before operation	50 c.c. –	25 c.c. –	———
After operation	Head and thorax circulation for 2½ hrs.		
		25 c.c. ±	———

had a suggestive color, but this was not striking. This fat was tested in the usual way for bile pigments with negative result (26 gm. of supraclavicular, 10 gm. of cardiac, and 32 gm. of omental fat, in separate specimens). The aorta is completely occluded by the clamp and the vena cava by the ligature. The spleen and viscera below the diaphragm show a good deal of engorgement.

The four preceding experiments give the final proof that the liver is not essential to a rapid change of hemoglobin into bile pigment. Of the four experiments one was not definitely positive but the others were positive in every respect and demonstrate that hemoglobin circulating actively in the head and thorax for a space of two hours will be in part changed to bile pigment. Not only the blood serum from the heart, but also the fat in the epicardial, mediastinal, intercostal, and supraclavicular regions contain readily demonstrable amounts of bile pigment. The zone of demarcation shows in the gradual change from the canary yellow fat of the thorax to the normal abdominal and peritoneal fat. No objections can be brought against these experiments on the ground of trauma and operative manipulation of the liver, for the organ was not disturbed at any time and the bile pigments cannot be derived from injured liver parenchyma.

It will be seen from this and the preceding paper that hemoglobin has been introduced into the circulation of dogs, first normal, second with Eck fistula, third with Eck fistula and hepatic artery ligation, fourth with exclusion of the liver, spleen, and intestines, and lastly with head and thorax circulation. This gives a series in which the liver circulation diminishes from normal to zero and the same ratio must hold for its physiological activity and participation in the systemic reactions. The change of hemoglobin to bile pigment goes on in much the same way and in about the same time under these different experimental conditions, from one to two hours being necessary for the elaboration of bile pigment. It must be remembered that it is easier to detect bile pigment in the urine than in the body fluids and tissues.

What tissues or cells may be concerned in this reaction? In the head and thorax circulation experiments one may imagine the change to be effected by (1) endothelium, (2) bone marrow, or (3) supporting tissues or muscle. There is evidence that the endothelium

may be the active factor. It is known that endothelium is capable of engulfing and digesting red cells and in certain diseases this activity is striking. The bone marrow cannot be ruled out as a factor. One possible way of settling this point is by means of a heart and lung circulation, and we hope to report further on this subject.

Those who believe that the liver is necessary for the formation of bile pigment may support the theory that the liver elaborates a ferment, present in the blood, which brings about the change of hemoglobin to bile pigment. Experiments with blood serum, hemoglobin, and various organ extracts *in vitro* do not bear out this theory, but cannot be said definitely to disprove it.

SUMMARY.

The intravenous injection of red cells obtained from the same animal and laked by distilled water is similar to certain types of hemolysis which result in hematogenous jaundice. This procedure cannot be criticized on the grounds of introducing toxic substances. The hemoglobin circulating in the blood stream is rapidly changed, in part at least, to bile pigment. The change goes on with practically the same rapidity in a normal circulation, in an Eck fistula animal, and in a dog with Eck fistula and hepatic artery ligation. Moreover, the bile pigment formation goes on in a dog whose liver, spleen, and intestines have been shut out of the circulation, and in those with a head and thorax circulation. In the last experiments there had been no operative manipulation of the liver and the bile pigment could not have escaped from the liver and have been absorbed by the circulation above the diaphragm; for example, by the thoracic duct. It is possible that the endothelium of the blood vessels is the agent which brings about the rapid change of hemoglobin to bile pigment. This mechanism probably comes into play when there has been a destruction of many red cells with much hemoglobin free in the plasma. We may conclude that in dogs, at least, hemoglobin can be rapidly changed into bile pigment in the circulating blood without participation of the liver.

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MORPHOLOGICAL STUDIES IN EXPERIMENTAL CRETINISM.*

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In making a study of the physiology of experimental cretinism,[1] suggested to me by Professor A. J. Carlson, of the University of Chicago, a large amount of material became available for morphological observations, and it is with this phase of the subject that the present paper is concerned.

Several members of litters of very young rabbits, from two to three weeks old, weighing from 150 to 250 gm. each, were thyroidectomized, while at least two members of each litter were kept for controls. All were weighed at regular intervals for the charting of growth curves. The operative technique of Hofmeister was followed, in the main. A median incision of about 1.5 cm. in length was made to center over the prominence of the larynx. With fine pointed forceps the inferior pole of the thyroid was freed from the laryngeal nerve, then carefully separated upward. The single thyroid artery is either ligated before division, or crushed with the forceps till coagulation within allows a division without ligation. This last procedure was very satisfactory in cases of young rabbits with relatively small arteries. The deeper superior pole was followed laterally and superiorly till its sheath was exposed, and was then separated from the connective tissue. After freeing the median superior attachment from the thyroid cartilage, this lobe together with the connecting isthmus was reflected over the remaining lobe, which was separated in a similar manner. Thus the whole thyroid apparatus was removed. At the superior poles of the glands, considerable difficulty was encountered on account of the intimate relation of the thyroid capsule to the thyroid cartilage. As is well known, the external pair of parathyroid glands are lateral, or sometimes inferior to and separate from the thyroid lobes. These, while usually seen during the operation, were rarely touched or disturbed in any manner. The whole operation, done aseptically, and under light ether anesthesia, rarely required more than one half hour. The operative procedure at the age of the animals used was well borne, and in no way influenced subsequent growth. This is shown

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[1] This work was undertaken primarily to establish a biological test for the active principle of the thyroid glands. The preliminary work was largely done in the Hull Physiological Laboratory of the University of Chicago.

by the growth curves of the rabbits which at exploratory examination, or at autopsy, revealed various sized nodules of thyroid tissue at the superior line of attachment of the thyroid capsule to the cartilage. These animals grew as rapidly and normally as the controls, and demonstrated, in addition to the innocuousness of the operation, the fact that surprisingly small nodules of hyperplastic glands, under these conditions, fully supplied the physiological need.

The completeness of the thyroid removal could never be absolutely determined at the time of the operation. Yet, after a few weeks, certain diagnosis of the animal's thyroid apparatus can be made from the growth curve and general appearances. While the term cretinism has been applied to the condition found in congenital absence of functioning thyroid, and while some may criticize the application of the term to a postnatal operative thyroprival cachexia, the rabbits are essentially cretins, showing great depression of rate of growth, are of pot belly type, with scaly skin, coarse thin hair, and are quite slow and awkward. They fully satisfy the description of cretin rabbits as given by Moussu, Gley, Hofmeister, and Haushalter and Jeandelize. Leonhardt apparently failed in all, while Blumreich and Jacoby failed in several cases, to effect a complete thyroid removal. This is apparent from a study of the increase in weights of their animals after thyroidectomy. Hofmeister obtained several cretins. He does not state how many failures he had, but he does state that not all of his thyroidectomized rabbits developed into cretins. Moussu, and Haushalter and Jeandelize described two or three cases each, and from reading their reports one is led to believe that no difficulty is to be encountered. Bensen operated only on young adult rabbits, and while his series is not strictly comparable to that reported in this paper, he nevertheless obtained definite morphological changes in certain organs.

The following protocols are taken from a series of about twenty-five autopsies, and comprise only those of completely thyroidectomized animals. The histological findings, which are constant and characteristic, will be given in detail in a single case, and in abbreviated form in the remaining eleven cases. None of the animals was killed for the purpose of morphological study. A large number were autopsied immediately after spontaneous death, following a rapid fall in temperature, and apparently due to the cachectic condition of the animals. Two or three were accidentally suddenly

killed by intravenous injections of normal defibrinated rabbit blood, which was first filtered through sterile gauze to remove clots. The histological picture of these animals differed in no essential respect from those dying spontaneously. For histological controls unthyroidectomized rabbits of corresponding ages were used. The tissues were all fixed in formalin, hardened in alcohol, and stained with hematoxylin and eosin.

PROTOCOLS.

Rabbit 54.—Albino, female; weight, 200 gm. December 9, 1911. Thyroidectomized. Weight of control 54 A, 193 gm. January 4, 1912. Weight, 558 gm. Weight of control, 814 gm. The hair of the cretin is ruffled and falling out badly; skin, scaly and dry. The abdomen is distended, and of the pot belly type. The animal appears to have a good appetite, yet moved about but little. April 6, 1912. Weight, 879 gm. Weight of control, 2,011 gm. Nothing unusual was observed. April 7, 1912. Reported dying by attendant. Autopsy was made while the body was still warm, within one half hour after death.

Autopsy.—The skin is dry and scaly, and the subcutaneous tissues are moist and glistening. The abdomen is distended, but there is no gas in the intestines. 2 c.c. of clear peritoneal fluid was obtained which coagulated within fifteen to twenty minutes to a cloudy, firm clot. No signs of infection or peritonitis. The lungs are congested, the liver is pale in color, and on section the kidneys seem to be congested. The thymus is of good size. The ovaries are large. The parathyroids are pale. There are no macroscopic signs of thyroids. The cerebral meninges are congested and the hypophysis cerebri enlarged. The bone marrow is firm.

MICROSCOPIC EXAMINATION.

Heart.—The myocardium presents a remarkable picture of degeneration of the individual muscle fibers. The fibers, in cross section, appear throughout to consist of a single layer of peripheral sarcostyles surrounding a central clear area which may or may not contain a nucleus. The muscle cells have not only lost all but the peripheral layer of sarcostyles, but in addition are swollen to two or three times the size of the normal heart muscle fibers. In areas this swelling is not marked, yet the central sarcostyles have disappeared, being replaced by clear non-staining areas. There is no indication of fatty degeneration. In longitudinal section of the fibers, the fibrillæ are very prominent; the longitudinal striation of the fibers being especially marked, while at the same time appearing to consist of rows of minute granules. These areas of the section also reveal the shell character of the fibers. The cytoplasm takes a good stain with eosin, and the nuclei are also well stained and normal in appearance. There is edema of the interfibrillar tissue.

Lungs.—The pleura is normal. The arterioles and capillaries are congested, while the larger arteries are empty. The veins are greatly distended. The bronchioles are practically all collapsed, the columnar epithelium of which is uneven, ragged, granular, vacuolated, and partly desquamated. The alveoli in many areas particularly about the bronchioles are atelectatic, while those about the periphery are emphysematous.

Liver.—The intimal walls of some of the central veins are thickened, with dilated lumina. Connective tissue about the bile ducts is loose and wide. The liver cells are, for the most part, two to four times as large as the normal liver cell. In scattered small areas are found cells of about normal size and appearance. The larger cells are paler in color, somewhat transparent, with relatively few, very large coarse granules in the non-staining matrix of the cells. Some appear to be confluent, with no separatory walls. Nuclei occur largely in pairs, are large, and sharply stained. The largest and palest cells have pale nuclei, or none at all. No fatty degeneration is shown. The cells of the section, as a whole, have a washed out appearance.

Spleen.—A condition of hyperplasia of pulp tissue and hypoplasia of Malpighian corpuscles is found. There are a great many large and small phagocytes in the pulp sinuses, the larger of which are filled with cell fragments and hemosiderin granules. The spleen is also congested.

Pancreas.—The secretory parenchyma appears normal and sharply staining. The islands of Langerhans are very distinctly marked off from the rest of the tissue, and are made up of an increased number of enlarged, well staining cells. The nuclei vary from large vesicular to small and dense types.

Kidney.—The capsule is normal. The larger blood vessels are surrounded by loose areolar tissue of decidedly edematous appearance, which immediately about the vessels takes a pale blue stain. The glomerular tufts are congested; the glomerular epithelium is well stained, yet vacuolated in some areas and granular in others. The nuclei are sharply stained. The cortex shows scarred areas in which the parenchyma is replaced by connective tissue. Nuclei of the cells of the tubules, are, for the most part, well stained, yet in some small areas are very pale, or absent entirely. Cytoplasm is decidedly granular and ragged. In the cells of certain of the convoluted tubules are dense and deeply eosin-stained droplets of colloid degeneration. Some vacuolation is also found in the cortex. The collecting tubules are lined by excessively swollen cells which exhibit but a nucleus and a sharp cell border, and are without demonstrable cytoplasm.

The transitional epithelium of the pelvis of the kidney and of the urinary bladder presents the same picture of swollen, clear, vacuolated cells.

Skin.—The horny layer is scaly, rough, and varies greatly in thickness. The corium is wide and loose, *i. e.*, edematous, without signs of mucoid appearance. The edematous corium grades rapidly into the dense subcutaneous tissue beneath. The blood supply is poor.

Esophagus.—The muscle shows some atrophy of fibers. Owing to edema, the areolar tissue between the muscle and epithelium is at least twice the normal width. Some fat cells are found in the areolar tissue. The epithelium appears normal. Several lymphoid nodules are found.

Large Intestine.—The individual cells of the outer muscular coat are separated from one another by spaces bridged across by cytoplasmic processes resembling protoplasmic bridges. Under oil immersion this appearance is found to be due to a continuous row of vacuoles encircling the periphery of the fiber. The muscle cells themselves are degenerated to a varying degree. Some, cut in cross section, are very pale and finely granular; others are dense and appear normal. The pale cells are usually very large, *i. e.*, swollen to two or three times the normal size.

The mucosa contains great numbers of goblet cells which, in the base of crypts, form a continuous layer.

Trachea.—The muscle is the same as in the esophagus. The areolar tissue between the cartilage and epithelium is abnormally wide and edematous. The blood and lymph vessels are much distended. The epithelium appears normal.

Ovary.—Cross section of the ovary measures 5 by 7 mm. Nine follicular spaces can be seen by the unaided eye. These are from 1 to 2 mm. in diameter. Germinal cortical epithelium is cuboidal or low columnar, the cytoplasm of which is for the most part vacuolated by serous imbibition. The nuclei lie towards the distal ends of these cells. Many of the cells composing the cords are vacuolated, others have clear undefined areas similar to those of the cortical epithelium. Primitive follicles are fairly numerous, but the nuclei of the ova are undergoing karyolysis. The large follicles make up a large part of the sections. There is relatively little stroma. Numerous germ hills are found in the large follicles, yet no normal ova are found in the entire section. In several of the medium sized follicles, the ovum is replaced by a disorganized cell mass in which are found bands of hyaline material apparently representing degenerated zonæ pellucidæ. In one instance, a complete ring of this material is seen to surround a mass of degenerating follicular cells in an early necrotic stage. The large follicles are filled, for the most part, by a thin, hyaline-like material. One small follicle is found filled with closely packed, small, round cells, which resemble lymphocytes.

Oviduct.—The stroma and muscle appear normal. The epithelial cells are coarsely granular and some indicate mucoid degeneration from the typical reaction with hematoxylin.

Mesenteric Lymph Glands.—Sections present the general picture of lymphoid hyperplasia. Numerous, isolated, fat cells are found in several of the lobes.

Adrenal Glands.—Cells of both the cortex and medulla are rather more vacuolated than normally. The ratio of the width of the medulla to the width of the cortex is greater than normally, apparently depending upon an absolute increase in the medullary area. Müller-formol fixation shows a large amount of chromaffin tissue. Cords of cortical cells interweave with cords of medullary cells at the boundary zone.

Bone Marrow.—The tissue is of the fatty type, with normal fat cells. Leucogenetic centers (Bunting) predominate over erythrogenetic centers. In the former the center is characterized by a few large myelocytes surrounded by polymorphonuclear leucocytes which show fragmentation and degeneration. Megalokaryocytes show generally karyolysis and cytolysis.

Parathyroid Glands.—The cytoplasm of most of the epithelial cells is poorly stained, *i e.*, chromophobic. At the same time definite vacuoles of nuclear size are plentiful. The cells are enlarged and swollen. There is no congestion, and no sign of either acinus formation or colloid.

Hypophysis Cerebri.—This organ shows three areas corresponding to the pars anterior, the pars intermedia, and the pars posterior. *The pars anterior* is rounded and constitutes by far the major part of the gland. The cells are grouped into clumps and cords. The majority of these cords take a light pink stain, while a small percentage are eosinophilic. The pale staining cells show in some cases definite vacuolation, in others irregular clear areas, with ragged boundaries. The *pars intermedia* is not sharply separated from the pars anterior,

but the cells are of a type similar to the eosinophilic cells of the pars anterior. Neither vacuolation nor appearance of neutrophilic or chromophobic character occurs in the cytoplasm. All cells in this zone are strikingly alike and constant. No colloid masses are found. *The pars nervosa* is infiltrated on its intermediary border by single cells or small groups of cells from the pars intermedia. The area of infiltration is somewhat pyramidal, with its base to the pars intermedia. On the border line between this infiltrated area and the nervosa proper is found a single duct, not unlike a single zymogenous pancreatic acinus in appearance. It consists of eight cells of low columnar or pyramidal type, with densely staining nuclei at the base of cells. The cytoplasm is finely granular and stains a deep pink with eosin. The pars nervosa, aside from a single doubtful instance, shows no sign of colloid. The exception is a small, nucleus-free, hyaline mass.

In order to avoid repetition, protocols of the rest of the series will be given in condensed form, except in cases of deviation from the types already described.

Rabbit 54 B.—Male. January 2, 1912. Thyroidectomized. Weight, 264 gm. Weight of control 59 A, 237 gm. April 22, 1912. Weight, 870 gm. Weight of control, 1,910 gm. This animal was unintentionally killed by an intravenous injection of 5 c.c. of defibrinated normal rabbit blood. Autopsy performed immediately. The litter of which this rabbit was a member had been raised in a hutch newly floored with heavy sheet zinc which became more or less polished by the rabbits. The thyroidectomized rabbits, 54 B, 55, and 57, developed muscular exhaustion in the adductors of the fore legs, so that they were unable to support themselves, but lay on their chests, turtle fashion, with legs extended laterally. The normal rabbits of the same litter and in the same hutch were not affected.

The skin is scaly and dry, and the hair is rough. The subcutaneous tissues are very pearly in appearance. The abdomen is distended. 8 c.c. of clear peritoneal fluid were collected. This coagulated to a firm, turbid clot. 1.75 c.c. of pericardial fluid were collected, which did not coagulate on standing. The heart is small, with a white dotted border at the base of the ventricles, also many small clear blisters are seen here. The auricles are still irritable and beating when dropped into formalin. The lungs appear normal. The thymus is small. The liver is spotted with brown flecks. The spleen is normal. The kidney pelvis is filled with a clear viscous material; the cortex is slightly congested. The adrenals and testes appear normal. The parathyroids are small. The bone marrow is dark and firm. Hypophysis measures 5.5 by 4 by 4 mm. Hypophysis of the control rabbit, which weighs 2,120 gm., measures 4.5 by 3.5 by 3 mm.

MICROSCOPIC EXAMINATION.

Heart.—Extreme sarcostyle degeneration, with edematous fibers.

Lungs.—Congestion, atelectasis, bronchial collapse, emphysema in areas. hemorrhage.

Liver.—Swollen, binuclear cells; slight karyolysis. No fat.

Spleen.—The pulp is hyperplastic, and there is an excess of pigmented phagocytes. Hypoplasia of the Malpighian corpuscles.

Pancreas.—The islands of Langerhans are hyperplastic and hypertrophic.

Kidney.—Granular degeneration of the cortical epithelium; granular degeneration and hydropic imbibition in the cells of the collecting tubules, pelvis, and urinary bladder.

Skin.—Edema of the corium. No mucoid stain.

Esophagus and Trachea.—The submucosa is edematous. The muscle shows two types of degeneration: (1) hyaline and (2) vacuolation.

Intestine.—Edema of the submucosa. Muscular degeneration. Vacuolation and swelling of the ganglion and sustentacular cells of Auerbach's plexus.

Mesenteric Lymph Glands.—Hyperplasia; fatty infiltration.

Adrenals.—Medullary hypertrophy; fatty cortex; congestion.

Testes.—Karyolysis of the germinal cells. No mature spermatozoa are found. The tubules are wide, but have a scanty cellular content. The interstitial cells of Leydig are large and plentiful.

Parathyroids.—The cells are vacuolated and somewhat swollen. No acini; no colloid formation.

Thymus.—Regressive type. Hassal's corpuscles are larger and more prominent than normally.

Hypophysis Cerebri.—The anterior lobe is congested. Fatty and serous vacuolation of cells. Some karyolysis. Relatively few eosinophiles. The cells of the pars intermedia are somewhat vacuolated. Neither colloid nor acinus formation is seen in any part of the whole organ.

Bone Marrow.—Fatty, inactive type; leucogenetic centers predominate over erythrogenetic centers. The megalokaryocytes are few and degenerating.

Striated Muscle (Pectoral).—Longitudinal striation is much more prominent than transverse striation; there are many areas of areolar tissue with only scattered single fibers, indicating absorption of muscle tissue. Some fibers show vacuolation, others are hyaline in character.

Cervical Adipose Tissue.—Shows serous atrophy.

Rabbit 18.—April 27, 1911. Thyroidectomized. Weight, 220 gm. October 24, 1911. Found dead. Weight, 930 gm. Typical cretin type.

Autopsy.—The hair and skin are typical. The subcutaneous tissues are pearly. 9 c.c. of clear, reddish, peritoneal fluid. Excessive pericardial fluid. The adrenals are larger than normally, 12 by 7 by 3 mm. There is no macroscopic thyroid. The hypophysis measures 6 by 4 by 3 mm.

MICROSCOPIC EXAMINATION.

Heart.—Extreme central sarcostyle degeneration.

Liver.—The cells are swollen. There is fat in the mid and central zones. Considerable hemosiderin.

Lung.—Atelectasis; bronchial collapse; slight emphysema; vascular dilatation.

Spleen.—Congestion. Malpighian corpuscles normal. Excess of pigmented phagocytes.

Kidney.—Parenchymatous degeneration. Hydropic vacuolation of the epithelium of the collecting tubules. The glomerular epithelium is vacuolated. Congestion.

Pancreas.—The islands of Langerhans are enlarged.

Skin.—The scaly layer is deeply stained with eosin. The corium is edematous and gives a mucoid stain with hematoxylin. Beneath this zone the blue color fades and passes gradually into the usual pink cytoplasmic stain of connective tissue.

Trachea and Esophagus.—There is marked edema in the submucosa which takes a distinct bluish stain about the esophagus. The muscle is vacuolated with edematous connective tissue separating the individual fibers.

Ovary.—Cystic degeneration of the follicles; karyolysis of the ova; germinal epithelium; hydropic.

Mesenteric Lymph Gland.—Slight lymphadenitis. The phagocytes are pigmented.

Adrenal Glands.—Hyperplasia of the medulla. The cortex is fatty, and there is also serous vacuolation.

Thymus.—The lymphoid elements are atrophic. Hassal's corpuscles are larger than normally.

Hypophysis.—Many fat and serous vacuoles are in the anterior lobe. There are numerous eosinophiles; no colloid formation.

Rabbit 44.—Female. August 21, 1911. Thyroidectomized. Weight, 245 gm. October 27, 1911. Died. Weight, 600 gm. Typical picture of cretin rabbit.

Autopsy.—The subcutaneous tissues are pearly. 10.5 c.c. of clear peritoneal fluid was obtained which clotted in ten minutes. The pleural cavity is free. The pericardium is filled with fluid. The thymus is undersized. The aorta is opaque. The spleen is small, and the kidneys are normal. The medullas of the adrenals appear larger than normally in proportion to the cortex. The bone marrow is pale.

MICROSCOPIC EXAMINATION.

Hypophysis.—Large numbers of eosinophiles are found in the anterior lobe. Both the anterior and intermediary lobes are vacuolated.

Esophagus, Skin, Aorta, and Kidney.—All show blue staining, edematous connective tissue.

Thymus.—The lymphoid elements are atrophic, and the epithelial elements hypertrophic. Three parathyroid nodules occur in this section, and are similar in character to the parathyroid previously described.

The other tissues are of the same type as those previously described.

Rabbit 48.—Female. Typical cretin; age, 7 weeks. Of especial note is the occurrence of a parathyroid nodule in the thymus; also an edematous corium and subcutaneous tissue with a mucoid reaction.

Rabbits 67 (age, 10 days), 80 (age, 5 weeks), 101 (age, 6 weeks), 55, 57 (age, 4 months), and 90 (age, 2 months), need no especial description, as they all show the typical macroscopic and microscopic appearances.

Rabbit 96.—Male; age, 5 months. Typical cretin. Hematoxylin and eosin sections are typical.

In order to ascertain the extent of fatty degeneration in the various tissues, tissues of rabbit 96 were stained with osmic acid. The microscopic analysis follows.

Heart.—Very fine black droplets were found along the fibrillæ of the shell, but not in the central area of the fiber.

Lungs.—Fat was found only in a few large oval cells.

Liver.—Fat droplets are rather evenly distributed throughout all the liver cells. Around some of the portal veins were small accumulations of fat droplets in the connective tissue cells.

Spleen.—A small amount of fat is diffusely spread throughout the organ.

Kidney.—A small amount of fat is found in scattered areas only.

Pancreas.—Scattered granules are found throughout except in the islands of Langerhans.

Testis.—Black droplets were found mostly in the epithelial cells, yet some cells were free from fat. A slight amount of fat was found in the perivascular connective tissues, and in certain extratubular cells.

Urinary Bladder.—The epithelial cells appear swollen, yet clear, with no signs of fat. The connective tissue immediately below was rich in fat, while none was found in the deep edematous connective tissue and muscle layers.

Intestine.—There is no fat in the muscle or ganglia, but considerable fat is found at the bases of the epithelium.

Adrenals.—The cortical cells are intensely fatty. There is no fat in the medulla.

The following is a summary of the constant morphological changes in the various organs, which characterize cretin rabbits.

Heart.—Swelling of the muscle cells with degeneration of the central sarcostyles, leaving a shell of external fibrillæ, which are degenerate in character.

Liver.—Fatty degeneration in the mid and central lobular zones, with often a great swelling of cells due to serous imbibition.

Kidney.—Fatty and granular degeneration and vacuolation of the cortical epithelium. Hydropic imbibition in the epithelial cells of the collecting tubules and ducts of Bellini; similar changes, also, in the epithelium of the kidney pelvis and urinary bladder.

Lungs.—Congestion; atelectasis; bronchial collapse; and emphysema.

Ovaries and Testes.—Incompleteness or absence of development of the sex elements with degeneration of the germinal epithelium.

Hypophysis and Parathyroid.—Swelling of cells, together with serous and fatty vacuolation. No acinus or colloid formation.

Pancreas.—Hypertrophy and hyperplasia of the islands of Langerhans.

Thymus.—Atrophy of the lymphoid elements, and hypertrophy of the epithelial elements (Hassal's corpuscles).

Muscle.—Serous infiltration, atrophy, and fatty degeneration of the smooth and striated muscle.

Bone Marrow.—Fatty, inactive type, with leucogenetic centers in excess of the erythrogenetic centers.

Adrenals.—Hypertrophy of medullas with plentiful chromophile substance; also excessively fatty cortices.

Body Cavities, Skin, and Connective Tissues.—Ascites; hydropericardium. Edema of the connective tissues generally is well marked in the true skin. This edema only occasionally gives a mucoid reaction with hematoxylin.

The degenerative changes in the myocardium are very pronounced. The individual cells are mostly somewhat swollen, and usually with but a single layer of external sarcostyles. The area of the undifferentiated cytoplasm, as described by MacCallum, is very small in the normal rabbit, and requires careful observation in order to be seen at all. In the heart cells of this series, the undifferentiated areas comprise a major part of the fiber. This may be due in part to a serous imbibition, associated with the central sarcostyle degeneration. The muscle in this condition cannot be otherwise than very weak, and forms a probable cause of the extreme dilatation and congestion of the lung vessels, and of the ascites and general tissue edema in the various organs. Although a similar degeneration of heart muscle of the rabbit is found as the result of other causes, yet in no case have I found such a general involvement of heart fibers as in these cretin rabbits. In other conditions, the degeneration is limited to small areas and borders, particularly those lining the cavities of the heart. Bensen, in studying the histology of thyroidectomized adult rabbits, and Kishi, in thyroidectomized cats and dogs, saw changes in the heart muscle, but laid no particular stress on such a condition. They found some granular degeneration, an increase in prominence in the longitudinal striation, with partial disappearance of the transverse striation. Bensen speaks of one case in which the fibrillæ were spread apart as if by edema, and in which a few fibers were devoid of nuclei, thus leaving a clear space. I have not studied cardiac muscle changes in thyroidectomized adult rabbits, dogs, or cats, but from the findings of Bensen and of Kishi it is evident that relatively slight changes occur. In contrast with this, my results indicate a very marked increased sensibility of the heart of the young rabbit towards the deleterious effect of athyroidism.

To what extent the degenerative changes in the liver may be due to cardiac insufficiency is difficult to determine. The marked

swelling of the cells, with thin cytoplasm and prominent granules, might be referable to a serous imbibition following venous stagnation, and in this manner the fatty degeneration of the cells of the central and mid zones might be explained, yet both lesions might be attributed to disarrangement of the internal metabolic activity of the cell dependent upon the absence of the thyroid secretion. Bensen found in some of his series a "*pflanzenzellenartiges Aussehen*" of the liver cells, and this condition is also described by Gozzi. This is essentially the same as in the present series. Rosenblatt found hyperemia and small hemorrhages, and some parenchymatous degeneration; while Hofmeister could see no constant abnormality. Halsted found the liver of a myxedematous dog to be normal. Since Rosenblatt and Halsted both used the dog, their series may not be comparable to mine, yet it might well be true that their different liver findings were due to a less affected condition of the heart.

The most characteristic feature of the kidney is the signet ring vacuolation of the cells of the collecting tubules and ducts of Bellini, also in the transitional epithelium of the urinary tract. There is also a marked parenchymatous degeneration and vacuolation of the epithelium of the convoluted tubules, and hydropic imbibition or vacuolation in the glomerular tuft epithelium. Colloid-like granules are found in the epithelium of the convoluted tubules. Parenchymatous degeneration was found by Bensen, Blum, Haskovec, Rosenblatt, D'Amore, Falcone, and Gioffredi. Hofmeister concluded that the only constant change was the peculiar hydropic imbibition of the cells of the collecting tubules. He had not studied the transitional epithelium of the urinary tract.

The amount of thymus tissue varied considerably in the different individuals. The lymphoid elements were atrophic, while the epithelial elements, or Hassal's corpuscles, were hypertrophic. Hofmeister, Haushalter and Jeandelize, and Blumreich and Jacoby, all found atrophic thymus glands, but did not notice any increased prominence of the epithelial portion. Blumreich and Jacoby state that the thymus atrophied in all of their series, even in those that showed no cachexia; and further, in some instances, they could find no trace of the thymus. I have always been able to find the thymus

in the cretin rabbit, but often care had to be exercised in order not to miss it. It must be recalled in this connection that the young adult rabbit has quite a large persistent thymus, and consequently the atrophic types, found in athyroid rabbits, is a prominent feature of the autopsy. The cause of differing responses of the two elements towards thyroid loss is obscure.

Atrophy of the chief parenchyma of the genital glands is a constant feature. An early atrophy of the thymus, according to Paton, facilitates the development and maturation of the sex glands, but in athyroidism there is non-development and degeneration of all but the interstitial cells of Leydig in the male. The male animals frequently showed some sexual desire, which indicates a fairly healthy condition of the interstitial element. Atrophic sexual organs were obtained in experimental athyroidism by Moussu in 1892. Hofmeister, Langhans, Lancereaux, Lanz, Dolega, Abrikossoff, and Halsted, made similar observations in different species of animals, including man. Halsted, in reporting an autopsy of a myxedematous dog, states that not only was there complete absence of spermatogenesis, but also almost complete absence of the cells of Leydig. The thymus in this animal was somewhat atrophic. In the ovary in my series there is in general marked karyolysis in the smaller ova, while the larger ones show complete absence of a nucleus, and in fact usually degeneration of the cytoplasm. The germinal epithelium shows the vacuolation so prevalent in other organs.

With respect to changes in the external parathyroids, I find no evidences of transformation into thyroid characteristics. The cells are enlarged, but are also vacuolated by a serous material. No evidences of colloid or acinus formation have ever been seen. Pepere states that he has observed changes indicating increased parathyroid function, deeper staining cells, some tendency to form acini, with colloid apparently arising from degenerating cells within the encircling cells. He claims, however, that these signs are due to increased parathyroid function following a partial parathyroid tissue loss, and are not due to any vicarious assumption of a thyroid function. Certainly my series gives no support to the theory proposed by Gley, Baber, and Horsley, and later supported by Vin-

cent and his associates. Gley and Nicolas, it will be recalled, later renounced the transformation theory. Vincent's criticism of such work is valid in so far as our operation is not a simple thyroidectomy, but also a removal of those internal parathyroids intimately associated anatomically with the thyroid lobes. Pepere called attention to the variability in size of these, that they were often extremely small, and hence the parathyroid loss associated with thyroidectomy in the rabbit removes a relatively small proportion of the whole parathyroid apparatus. It has been repeatedly shown that removal of one thyroid and the external parathyroid of the same side has no effect on the animal. In my operation, I have always preserved the external parathyroids intact, and have only observed cachexia thyropriva in those cases in which no thyroid residue was found. In some incomplete removals in autopsies not recorded here, there have been found quite large masses, sometimes five by five millimeters, of hyperplastic thyroid tissue, which from their location are apparently derived from some unremoved thyroid cells at the extreme upper attachment to the thyroid cartilage. It appears improbable that thyroid hyperplasia could only occur in those cases in which a supposed parathyroid nodule could be left in the unremoved portion. There remain, then, two alternatives: the external pair of parathyroids are unable to exert a sufficient vicarious thyroid function, and hence are of a different character from the internal pair, or, that cachexia thyropriva is only prevented by the function of thyroid tissue itself. No histological evidence has been presented to indicate any differences in character between the internal and external pair of parathyroids. Hence we are forced to believe that the thyroids and parathyroids are inherently separate and distinct organs.

The study of the hypophyses of my series reveals a constant enlargement, yet there are no sure signs of colloid, and absolutely no indication of any thyroid-like acinus formation. The presence of serous and fatty vacuolation together with frequent karyolysis indicates a degenerative process. Whatever this gland may have done to ameliorate the untoward effects of athyroidism, it certainly is unable to compensate fully for thyroid deficiency. Enlargement of the hypophysis of thyroidectomized animals was first shown by

Rogowitsch in 1889, then confirmed by Stieda, Tizzoni and Centanni, Gley, and many others. Hofmeister, and also Alquier, considered the enlargement to be compensatory towards the thyroid loss. Schönemann, in making a study of the relation of the thyroid to hypophysis in human autopsy material, came to the conclusion that the hypophysis became enlarged due to degenerative causes rather than to compensation. Simpson and Hunter could find no iodin in the hypophysis of their thyroidectomized sheep, and concluded that if an iodized substance represented the essential secretion of the thyroid that the hypophysis could scarcely be considered to compensate by this means. Guerrini, Gemelli, de Coulon, Ponfick, and Pirone, believed with Schönemann that the hypophysial enlargement was not necessarily a vicarious compensation, but may be produced by many intoxications, such as diphtheria toxin, snake blood, and toxins from intestinal ligation. The hypophyses of my series are distinctly degenerate in character and would scarcely warrant an assumption of vicarious compensation, more than could equally well be ascribed to the enlarged liver cells.

The bone marrow is usually of an inactive, fatty type, with the erythrogenetic centers exceeded somewhat in numbers by the leucogenetic centers. Thus there is indicated a decreased production of erythrocytes. That there has been no excessive leucocyte demand is shown by fragmentation of leucocytes accumulated about the borders of the leucogenetic centers. The megalokaryocytes are degenerate in type. Esser, in 1907, found the bone marrow of the thyroidectomized rabbits to be fatty in type, with degenerating megalokaryocytes. He believes the bone marrow to be responsible for the anemia of hypothyroid animals.

The spleen of the cretin rabbit appeared small, though no measurements were taken. The histological picture is relatively constant. There is an excess of pigmented phagocytes indicative of an excessive erythrocyte destruction in the organism. Hyperplasia of the pulp and occasionally sclerosis may be referable to an early congestion which was present in some of the series. The congestion and pigmentation were observed by Bensen and by Gozzi. Zezas reported a vicarious hypertrophy, which was negatived by the work

of Hofmeister and of de Guervain. Halsted found the spleen of the myxedematous dog to be normal.

The islands of Langerhans in the pancreas in this series were definitely hyperplastic and hypertrophic, and sharply defined, while the zymogenous tubule epithelium appeared normal. Most authors have failed to see any pancreatic changes in thyroidectomized animals, though Falta and Berterelli, according to Biedl, state an increase in size and number of the island cells in hypothyroidism. This condition has an interesting bearing on the question of increased sugar tolerance in cases of hypothyroidism. No evidences of any transition of zymogenous epithelium into islands of Langerhans could be seen. Both were sharply limited with no transitional types as described by Vincent.

The general condition of edema, ascites, and excessive pericardial fluid may be largely due to cardiac insufficiency, though it is not improbable that metabolic disturbances, as studied by Kottmann, may play an important rôle. The atelectasis and bronchial collapse seem explicable only on the assumption that the greatly widened pulmonary vessels following gradual cardiac failure have produced an increased intrathoracic pressure.

Both smooth and striated muscle show marked degeneration, the former giving rise to intestinal distension resulting in the pot belly symptom of all cretins; the latter explains the weakness of skeletal musculature.

SUMMARY.

In summarizing the findings of this paper it may be said that degenerative changes have been noted in practically every parenchymatous organ. Among these the most striking has been that of serous imbibition by the most active cells of these organs.

In regard to the changes in the glands of internal secretion, the findings corroborate the statements of Cushing in regard to hypophysectomy, that removal of one gland of internal secretion results in changes in all the other glands. In this case, degenerative changes predominate in the hypophysis, thymus, ovary, and testis, while hyperplasia is seen in the islands of Langerhans and the medullas of the adrenal glands.

Finally, in the rabbit athyroidism is responsible for grave degenerative changes in practically all organs and tissues of the body, and many of the symptoms of cretinism have an anatomical basis in organic cellular changes.

In conclusion the author wishes to express his appreciation of the assistance of Professor C. H. Bunting, at whose suggestion the histological material was studied, and in whose laboratory the material was prepared. The author also takes pleasure in acknowledging to Professor A. S. Loevenhart many helpful suggestions.

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A NOTE ON THE BEHAVIOR OF THE SAPROPHYTIC COCCI WITH REGARD TO GRAM'S STAIN.*

By I. J. KLIGLER.

(*From the American Museum of Natural History, New York.*)

C.-E. A., and A. R. Winslow[1] found that one of the fundamental differences between the parasitic and saprophytic cocci was their behavior to the Gram stain. The parasitic forms (Paracoccaceæ) generally retained the stain, while the saprophytic group (Metacoccaceæ) generally decolorized by Gram.

In my recent revision of the Coccaceæ in the collection at the Museum of Natural History,[2] I was able to corroborate the observations made by these authors. Of twenty-three strains belonging to the subfamily Metacoccaceæ, seventeen, or 74 per cent., were Gram-negative; two, or 9 per cent., were Gram-positive; and four, or 17 per cent., gave a variable reaction, giving both positive and negative tests either on the same slide or on different occasions. Three quarters of our strains thus appeared as Gram-negative, while only one twelfth of the cultures gave a repeatedly positive test. Winslow, using about 250 strains, obtained only 22 per cent. of Gram-positive reactions in this group.

While studying the parallelism between the Gram reaction and inhibition of growth by gentian violet, Churchman[3] tested a large number of our organisms, among which were most of our Metacoccaceæ. His findings proved entirely contradictory to mine, as all the cocci tested by him are without exception reported as Gram-positive.

Believing that the discrepancies were due to the differences in the

* Received for publication, March 29, 1913.

[1] Winslow, C.-E. A., and Winslow, A. R., The Systematic Relationship of the Coccaceæ with a Discussion of Bacterial Classification, New York and London, 1908.

[2] Kligler, I. J., *Jour. Infect. Dis.*, 1913, xii, (in press).

[3] Churchman, J. W., *Jour. Exper. Med.*, 1912, xvi, 221.

methods employed by us, I restained our cultures according to both methods. The techniques employed may be briefly summarized as follows:

Churchman's Method.

1. Agar cultures 24 hours old were used.
2. Aurococcus and *B. typhosus* were used as positive and negative controls, respectively.
3. Fixed as usual.
4. Stained with anilin oil-gentian violet for 2 minutes.
5. Washed with water and drained.
6. Gram solution for 5 minutes.
7. Decolorized with 95 per cent. alcohol for 3 minutes.
8. No counterstain was used.

Winslow's Method.

1. Young agar cultures, 3 to 5 days old.
2. No control was used at first. Later *B. subtilis* and *B. coli* were used as positive and negative controls, respectively.
3. Same.
4. Stained with anilin oil-gentian violet for 1½ minutes.
5. Same.
6. Gram solution for 1½ minutes.
7. Decolorized with 95 per cent. alcohol for 3 minutes.
8. Counterstained with a 1:20 solution of Bismarck brown in water, for 30 seconds.

The main differences lie in the time of staining, Churchman employing a longer period. It appeared probable, therefore, that the positive results obtained were attributable to the prolonged staining period. This, however, proved not to be the case, as may be seen from the results in table I.

The first two columns show the reaction of seventeen strains of saprophytic cocci stained by me according to both methods. For comparative purposes two columns are added, one giving the results previously obtained by me, using Winslow's method, the other showing the results obtained by Churchman. The table brings out the following facts: (1) The two methods employed lead practically to the same results. (2) The results obtained by me now vary only slightly from those obtained previously. (3) Churchman's results cannot be attributed to the difference in the staining period.

The different results obtained by Churchman and by me may perhaps be due to the fact that the interpretation of the effects of the Gram stain is notoriously difficult among the cocci and a number of other organisms. Many bacteria do not give the definite results that are characteristic of such forms as *Bacillus coli* on the one hand, and *Bacillus subtilis* on the other. Winslow says in this connection:

". . . All cultures of *B. coli* are decolorized by one minute's treatment with alcohol, while *B. megatherium* constantly fails to decolorize after three hours. On the other hand, with *B. fluorescens, B. diphtheriae* and certain cocci the result is affected by the time of decolorization, as well as by the age of the cultures. Between the fixed points at the extreme, preparations will yield varying results, showing some cells stained and others decolorized. As a rule, a large majority of cells in a given preparation will show one reaction or the other; but a second slide made from the same culture may yield a different result."[4]

TABLE I.[5]

Results Obtained by Employing Different Methods of Staining by Gram.

No. of strain.	Present results.		Previous results.	
	Method used.		Method used.	
	Winslow.	Churchman.	Winslow.	Churchman.
101	—	—	—	+
208	—	—	—	+
272	—	—	—	+
314	±	—	—	+
345	—	—	—	+
354	±	±	—	—
481	—	—	—	+
479	—	—	±	—
458	—	—	—	—
466	—	—	—	—
430	—	—	—	—
3	±	±	±	—
217	—	—	—	+
315	+	±	±	+
462	—	—	—	—
474	±	±	±	—
488	—	—	—	+

Churchman lays even greater emphasis on the uncertainty of the Gram stain which he claims cannot compare in precision and constancy with the gentian violet reaction. He says:

". . . the Gram stain is too notoriously uncertain . . . In numerous instances I have found great difficulty in deciding, even after repeated stains, whether to classify an organism as Gram-positive or negative; and a few discrepancies will be found in the table between the commonly accepted statements on this point and my findings."[6]

There is no doubt that the Gram stain often fails to yield as clean cut results as we should desire. Churchman has introduced a new

[4] Winslow, C.-E. A., and Winslow, A. R., *loc. cit.*, p. 49.

[5] The observations in the first three columns were made by me; those in the last column, by Churchman.

[6] Churchman, J. W., *loc. cit.*, p. 227.

test of great value in the gentian violet plate, and on this medium all the cocci appear to behave as Gram-positive organisms usually do; that is, they fail to grow in the presence of the dye. At the same time, in view of the results reported above, it seems justifiable to conclude that the Gram stain as ordinarily used is sufficiently constant to have a distinct diagnostic value; that there is a real difference in susceptibility to the Gram stain between the Paracoccaceæ and the Metacoccaceæ; and that the latter may be classed as usually Gram-negative organisms.

STUDIES UPON EXPERIMENTAL PNEUMONIA IN RABBITS.

Parts I to III.*

By M. C. WINTERNITZ, M.D., and A. D. HIRSCHFELDER, M.D.

(*From the Pathological Laboratory and the Physiological Laboratory of the Medical Clinic of Johns Hopkins University, Baltimore.*)

Plates 108 to 111.

I. The Production of Lobar Pneumonia.

In the course of some experiments carried out with Dr. H. M. Evans upon the action of benzol on the hematopoietic system, one of the rabbits that died showed a spontaneous pneumonia, lobar in character. The inflammatory exudate in the alveoli of the lungs presented a striking appearance: the cells of the exudate were not only the usual red and white blood cells, but also granulocytes and megakaryocytes, apparently arising from the bone marrow. This finding started us on an investigation to determine whether it was possible to produce lobar pneumonia in rabbits with any degree of uniformity.

Many of the older workers upon pneumonia (Fraenkel, Gamaleia, Weichselbaum, Salvecli, and Tschistovitch) produced occasional lobular or even lobar pneumonias by intratracheal inoculations of pneumococcus cultures in dogs, cats, and rabbits, and Monti claims to have produced typical fibrinous pneumonia of one lobe or an entire lung often complicated by pericarditis and sometimes almost without any general infection. None of these observers, with the possible exception of Tschistovitch and Monti, were able to produce the lesion with any degree of uniformity. Wadsworth, in 1904, came to the conclusion that in the rabbit "the development of acute exudative pneumonia offers an especially clear example of the nice balancing of the essential conditions determining infection," and stated that that was as yet for practical purposes beyond experimental control. Later, however, Lamar and Meltzer demonstrated, in 1910, that it is possible to produce lobar pneumonia uniformly in dogs by the intratracheal injection of large quantities of broth cultures and the pneumococcus. The low mortality (16 per cent.) of the disease in dogs and the difficulty of handling a large number of large animals simultaneously in most laboratories places ob-

* Received for publication, March 18, 1913.

stacles in the way of many forms of experimental investigations which are obviated by the use of rabbits.

The method employed in these experiments is essentially the same as that introduced by Lamar and Meltzer for the production of lobar pneumonia in dogs. In this series rabbits were used exclusively. The animals were etherized deeply, the mouth was held open by a simple instrument, and a small, soft, rubber catheter, three millimeters in diameter, open at both ends, and stretched over a silver probe to give it rigidity, was introduced through the mouth and larynx into the trachea. The silver probe was slightly bent to correspond to the curve of the larynx and was introduced with its concavity upward. The presence of the catheter in the trachea was easily determined by palpation. The procedure entails some difficulty. Under no circumstances must the slightest force be used, as injury to the tissues readily leads to hemorrhage into the trachea which usually results in the immediate death of the animal. The catheter must be inserted well down into the thoracic portion of the trachea. The probe is then removed and four to five cubic centimeters of culture fluid are injected from a syringe, after which the syringe is detached, the piston drawn out, and an equal volume of air is in turn forced through the catheter.

The culture employed was a typical pneumococcus obtained from the heart blood of a patient who had died of acute lobar pneumonia. It had been grown by Dr. P. W. Clough for several weeks and its virulence was kept high by repeated passages through white mice and rabbits. Twenty-four hours before inoculation it was transferred from blood agar to broth of double the usual strength containing one part of pig serum to twenty parts of broth, and sterilized on three successive days with moist heat. It has been found subsequently that frequent passage through animals is not necessary to maintain the virulence of the cultures, nor does it make any appreciable difference whether twenty-four or forty-eight hour cultures were employed.

One hundred and five rabbits were used. In twenty the disease was allowed to run its course uninfluenced by other experimental procedure. All the rabbits except three died; three died in less than twenty-four hours, four in twenty-four to thirty-six hours,

four between thirty-six and forty-eight hours, four between forty-eight and seventy-two hours, and two later than seventy-two hours.

In eighty-one animals the gross lesions were as follows: Consolidation of the entire right lung occurred nine times; of the entire left lung, nineteen times; of the upper right lung, six times; of the middle right lung, eleven times; of the lower right lung, twenty times; of the upper left lung, thirteen times; of the lower left lung, sixteen times; of both lungs, six times. Marked dry pleurisy occurred in thirty-five instances; effusion in sixteen; mediastinitis in ten; pericarditis in nine (figures 1, 2, 3 *a*, and 4 *a*). There was no consolidation in one case. This animal died thirty days after inoculation, and autopsy revealed a chronic fibrous pleurisy and encapsulated pleural and pulmonic abscesses. For five days after incubation this animal had the clinical signs of pneumonia including complete chloride retention for three days. Recovery seemed to take place by pseudocrisis.

Cultures were made in twenty-four cases, and in every case where they were made both the heart blood and lung yielded pure cultures of pneumococci. Cultures made during life from the ear vein by allowing blood to run from the ear vein on to slant agar invariably showed pneumococcemia.

Clinically the animals recovered from the operative procedure almost immediately and remained well for a variable period of time. They then began to look sick, refused food, had marked dyspnea, and in some cases they supported the affected side by resting it on the fore leg or bent the body to lessen the movement. It was sometimes possible to make out tubular breathing over the consolidated area. Pain could be demonstrated occasionally by a respiratory squeak elicited through pressure on the affected side; the white blood cells showed a persistent fall from the normal (5,000 to 8,000) to a level ranging from 2,000 to 4,600, and in one case they fell to only 760 per cubic millimeter. The temperature rose from normal (38.7° to 40° C.) to between 41° and 42° C. During the disease the chlorides were present in relatively large quantities in the first few days, curds with silver nitrate plus nitric acid having been given, but in two cases they were almost entirely absent from the third to the seventh day of the disease. In one

of the animals that recovered the chloride excretion returned to normal.

In order to demonstrate that the production of lobar pneumonia was not peculiar to the strain of pneumococcus used, another culture[1] was also tested. The results were identical with those recorded above.

II. Pneumonia in Animals Rendered Aplastic.

The experiments of Selling have shown that leucocytes may be caused practically to disappear from the circulating blood, and the bone marrow cells may be destroyed by the injection or inhalation of benzol. This method offered a ready means of studying the inflammatory exudate and the processes of phagocytosis and immunity in more or less aplastic animals. While this general problem had naturally arisen in the course of Dr. Selling's work, the present series of experiments was undertaken as a direct result of the accidental finding of an instance of spontaneous pneumonia in a rabbit in which bone marrow changes had been produced by the injection of benzol, and which was being studied for another purpose by one of the writers in collaboration with Dr. Evans. The pneumonic exudate contained, besides the usual elements, megakaryocytes and granulocytes corresponding to those formed in the regenerated hyperplastic marrow of the femur of the same animal.

From the finding in this animal it seemed probable that the nature of the cellular inflammatory exudate might depend upon what cells the marrow could supply at the time the demands of the body were made upon it, and that the cellular exudate might be varied by changing the absolute and proportionate number of the marrow cells, as may be readily accomplished by the injection of benzol. The rôle of phagocytes in the resistance of the animal, as well as many other studies, such as immunization in partially and completely aplastic rabbits, likewise suggested themselves for investigation by this method.

Several series of animals were injected with benzol. 1 c.c. of benzol per kilo of body weight, mixed with equal quantities of olive oil, were injected subcutaneously daily until the desired degree of leucopenia was attained. Pneu-

[1] This culture was sent us by Dr. J. O. Hirschfelder of San Francisco.

monia was produced in animals in many stages of aplasia and regeneration. This was accomplished by allowing varying intervals to elapse between the last dose of benzol and the production of pneumonia. It was found by Selling that animals injected with benzol after the leucocytes were 800 or less per cubic millimeter invariably died. If the injections are stopped at this stage the leucocytes will usually continue to fall for a variable period and then regeneration will ensue, provided the leucocytes have not fallen below 100 per cubic millimeter, when in our series death always ensued.

Pneumonia was produced in eight aplastic animals. In two the leucocytes were falling, in two they were stationary, and in four rising. At the time of infection the counts showed 280, 320, 400, 500, 850, 880, and 3,600 per cubic millimeter, as compared with 4,400, 6,400, and 7,500 in the controls. Counts made six hours after inoculation showed no important change in the number of white blood cells, either in the normal animals or in those treated with benzol.

The resistance of these aplastic animals to pneumonia was strikingly reduced. All the animals died in from thirteen to twenty-seven hours after inoculation, the average length of survival being twenty hours, while none of the six controls inoculated on the same days with the same dose of the same broth suspension of the organism died under twenty-five hours, the average being sixty-one hours.

The description of the consolidation and gross appearance of the involved portion of the pneumonic lung was exactly the same as in the normal rabbits. Pleurisy and pericarditis were not so frequent.

The histological appearances of the exudate varied with the degree of aplasia of the marrow. In the most highly aplastic animals, where there were only a few small islands of regenerating cells in the femoral bone marrow, the pneumonic exudate was likewise poor in leucocytes. It contained the usual number of red blood cells and the usual quantities of fibrin, but only occasionally polymorphonuclear leucocytes or undifferentiated mononuclear cells (figures 3 *b*, 4 *b*, 5 *a*, and 5 *b*). On the other hand, large numbers of pneumococci filled the alveoli. They were not clumped, but were diffusely scattered and were so numerous as to produce a fine stippling throughout the sections, though in the controls very few cocci

were visible (figure 5 *b*). The few white cells present, as well as the lining cells of the alveolar epithelium, contained pneumococci.

In the animals whose marrow showed more marked regeneration the exudate contained, besides the elements above noted, mononuclear cells exactly like the mononuclear cells of the marrow. Occasional large, partly degenerated cells were seen suggesting the megakaryocyte of the marrow, but, as a rule, these were so far degenerated that they could not be identified. The cell picture of the exudate corresponded to that of the marrow irrespective of the total number of white blood cells in the circulation.

This markedly decreased resistance of the aplastic rabbits to the pneumonic infection, together with the striking overgrowth of the pneumococci in the lungs of the animals seemed in harmony with Metchnikoff's theory of phagocytic immunity. The agreement was further borne out by the fact that a small series of animals treated with toluol in a similar way and in a similar physical condition, but whose marrow and blood count were normal, reacted to the pneumonic infection exactly like normal rabbits. This observation is being continued in collaboration with Dr. Kline.

III. Intra Vitam Staining.

The possibility of injecting anilin dyes into living animals and obtaining definite staining of certain tissues dates from Ehrlich's work in 1885. Ehrlich succeeded in staining most of the organs by means of indophenol and alizarin-blue. He was even able to differentiate normal areas from those which had undergone pathological changes, and states that in the hearts of animals with myocarditis, after injection of indophenol, the scars stand out blue against the unstained heart muscle. In the last few years, through the work of Ehrlich and Shiga, Nicolle and Mesnil, Bouffard, Fischel, Arnold, Michaelis, Goldmann, and others, a number of other dyes have been discovered which are also successful vital stains. In these instances the dyes are localized in the form of granules in definite cells. Until recently blood cells have not been stained *intra vitam*. Schulemann, however, demonstrated the presence of large vitally stained macrophages in the circulating blood, an observation made independently by one of the writers in collaboration with Dr. Evans.

The latter authors also showed that it was extremely rare for a polynuclear or other normal white blood cell to contain stained granules as long as it was alive, but, on the other hand, by killing the white blood cell in various ways they could be made to absorb

rapidly the vital stain. With this fact in mind, and since the pneumonic exudate consists of white blood cells, many of which are presumably injured or dead, pneumonia was induced in a few animals which were stained *intra vitam* in the course of the infection by intravenous injection of trypan-red or trypan-blue. A variety of dyes of the triphenylmethane series were used, but contrary to the statements of Morau and Germain See, they did not give rise to vital staining. The intravenous injection of trypan-blue and trypan-red gave rise to the usual diffuse staining as described by Bouffard, Goldmann, etc., but in addition to this the diseased area of lung showed a much more intense staining than any of the other tissues, while the normal lung tissue was practically normal in color. Particularly intense was the stain in the fibrinous exudates, not only over the lungs but on the pleura and pericardium and in the mediastinum. With varying amounts of the dye the intensity of the stain could be altered. With trypan-blue, for instance, minimal doses stained the fibrinous exudate a pale blue, while with larger amounts every gradation up to a royal blue could be produced. With the doses which gave the faintest stain in the exudate the tissues elsewhere were almost unstained, except the kidneys which showed about the same intensity of stain as the inflammatory area. There was thus a certain specific affinity manifested between the exudate and the dye. Histologically the stain was found to be located in the bundles and strands of fibrin of both serous and alveolar exudate, while the cells of the exudate were unstained except in a few instances. These cells showed staining of the nuclei but usually no intracellular granules, and were evidently dead cells. It is therefore evident that in these cases of early though extremely virulent experimental pneumonia almost all the leucocytes of the exudate were living. The animals studied, however, either died or were killed early in the course of the disease and quite possibly many more of the leucocytes die at a later stage in the course of more chronic infections.

An iodin derivative of trypan-blue prepared by one of us for another purpose, whose molecule had absorbed five atoms of iodin, differed slightly from the original substance in being of a deep

lavender rather than of a blue color, but it did not possess *intra vitam* staining properties.

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[2] Since writing this article our attention has been called to a paper by Rasquin. (Études expérimentales sur la pathogénèse de la pneumonie chez le lapin. *Arch. de méd. expér. et d'anat. path.,* 1910, xxii, 804) upon the production of lobar pneumonia in rabbits. Rasquin, however, states that he was unable to produce pneumonia in more than 8 per cent. of his rabbits unless the culture injected was mixed with the serum of dogs immunized to rabbit blood, in which case he obtained pneumonia in over 90 per cent. of his animals. The serum of normal dogs had no effect. Rasquin's results are, therefore, more comparable with those of Wadsworth than with our own. The results of Neufeld and Ungermann (Ueber experimentell erzeugte Pneumonien und ihre Beeinflussung durch Antipneumokokkenserum, *Centralbl. f. Bakteriol., Ref.,* 1912, liv, Suppl., 71), who have produced experimental pneumonia in the guinea pig by the intrapulmonary (not intratracheal) method also reached us after our work was completed.

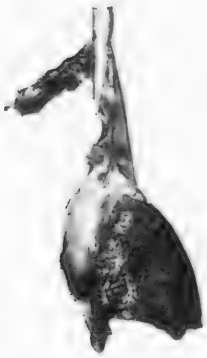
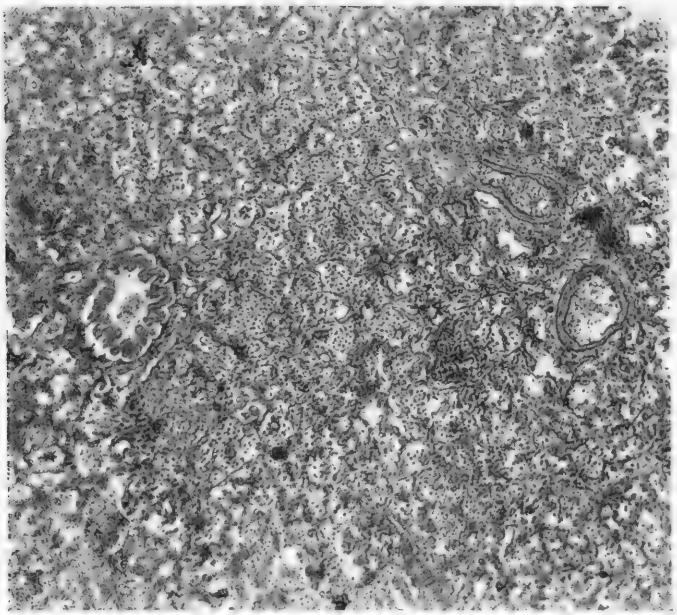


FIG. 1.

FIG. 2.

(Winternitz and Hirschfelder: Experimental Pneumonia



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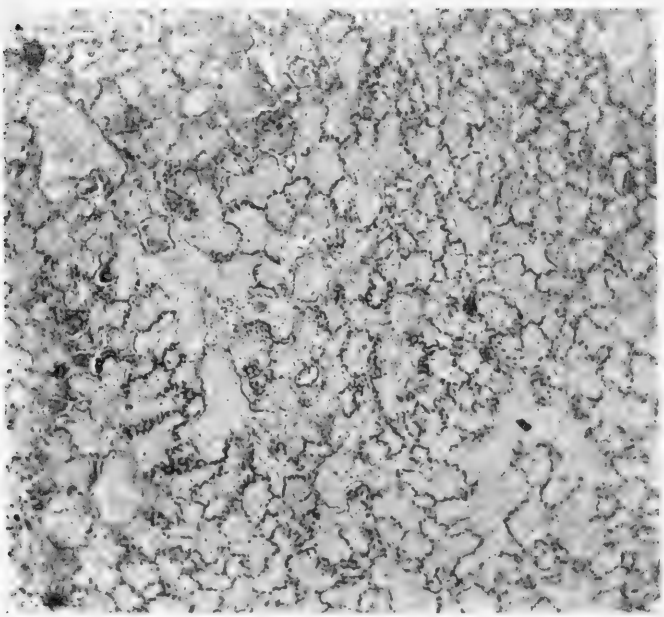

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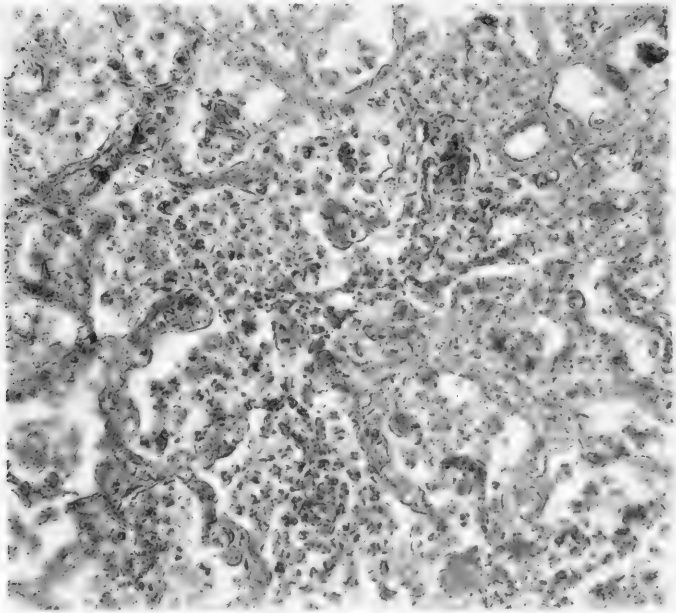
FIG. 3.

(Winternitz and Hirschfelder: Experimental Pneumonia.)



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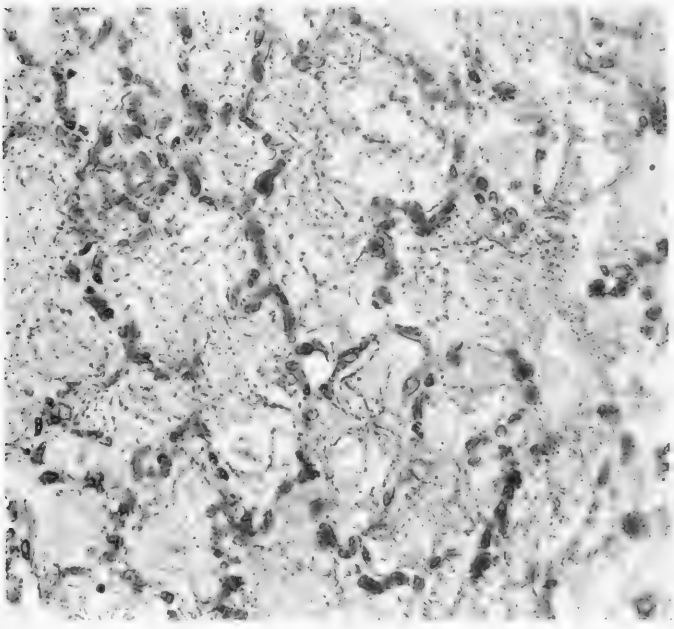

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FIG. 4.

(Winternitz and Hirschfelder: Experimental Pneumonia.)

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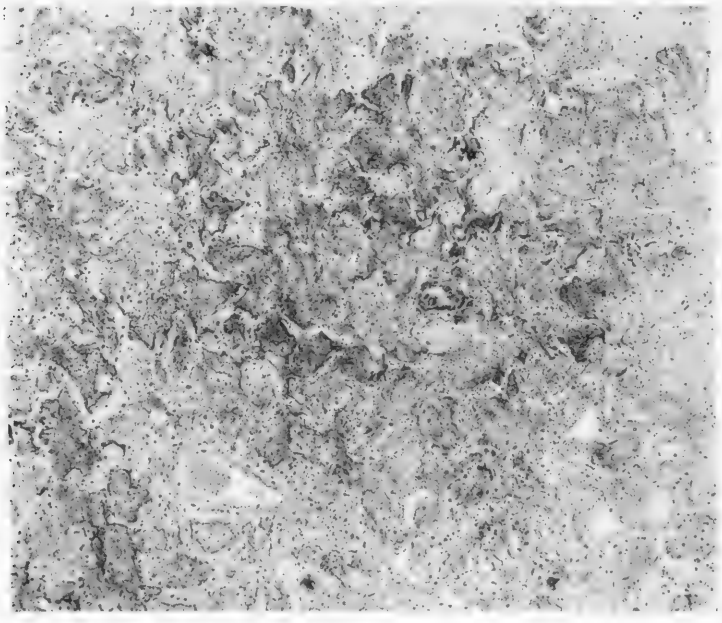

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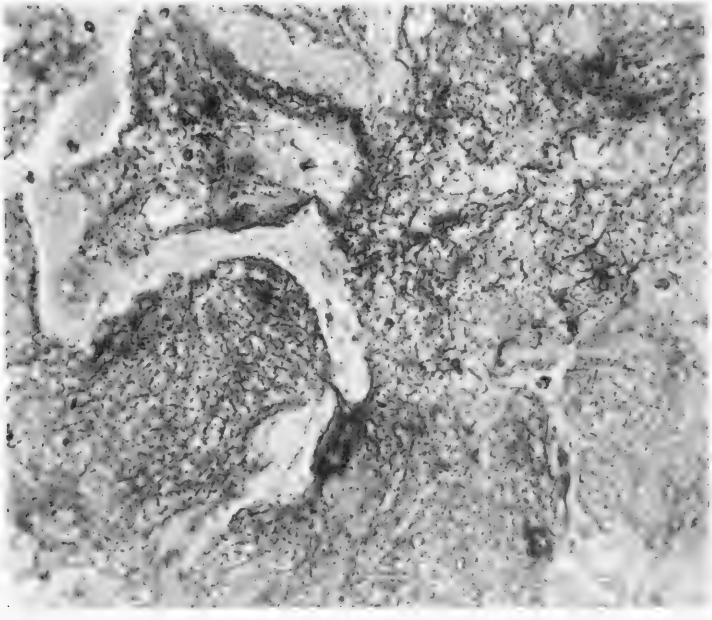

b

FIG. 5.

(Winternitz and Hirschfelder: Experimental Pneumonia.)

[illegible]

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EXPLANATION OF PLATES.

Plate 108.

Fig. 1. Experimental lobar pneumonia and fibrinous pleurisy in a rabbit's lung, showing fibrinous pericarditis as well.

Fig. 2. Experimental lobar pneumonias in a series of rabbits, the result of one day's experimentation, showing lobar consolidation in every case.

Plate 109.

Fig. 3 *a*. Section of lung of a normal animal with experimental lobar pneumonia. Low power.

Fig. 3 *b*. Section of lung of an aplastic animal with experimental lobar pneumonia, showing the paucity of cells in the exudate.

Plate 110.

Fig. 4 *a*. Section of lung of a normal animal with experimental lobar pneumonia. High power.

Fig. 4 *b*. Section of lung of an aplastic animal with experimental lobar pneumonia. The pneumococci are shown within the alveoli. High power.

Plate 111.

Fig. 5 *a*. Fibrin stain of a section of lung of an aplastic animal with experimental lobar pneumonia. Low power.

Fig. 5 *b*. Fibrin stain of lung of an aplastic animal with experimental lobar pneumonia. High power.

STUDIES UPON EXPERIMENTAL PNEUMONIA IN RABBITS.

IV. Is There a Parallelism between the Trypanocidal and Pneumococcicidal Action of Drugs?*

By A. D. HIRSCHFELDER, M.D., and M. C. WINTERNITZ, M.D.

(From the Physiological Laboratory of the Medical Clinic and the Pathological Laboratory of Johns Hopkins University, Baltimore.)

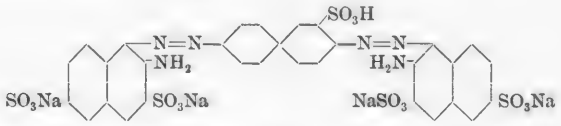
Morgenroth and his collaborators have shown that derivatives of quinin and cuprein, and especially of hydroquinin and hydrocuprein, possess therapeutic powers against both trypanosomes and pneumococci. In order to determine whether a general parallelism exists between the trypanocidal and pneumococcicidal action, experiments were carried out to investigate the effect of the various other groups of drugs that possess chemotherapeutic properties for trypanosomiasis.

Our investigations upon the vital staining of the pneumonic exudate have shown that the benzidin dye, trypan-blue,

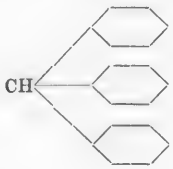
which Nicolle and Mesnil had shown to be a specific for experimental trypanosomiasis, has the power of selectively staining the pneumonic exudate. Since the drug is thus concentrated in the diseased area, we were led to the hope that if this parallelism existed it might be particularly well shown by this substance. Unfortunately such was not the case, and no marked difference could be distinguished between the vitally stained animals and the controls. Indeed, doses which had no deleterious effects upon normal animals seemed to hasten death in those with pneumonia. Equally

* Received for publication, March 18, 1913.

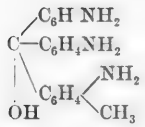
negative were the results obtained by using the iodin derivative of trypan-blue prepared by one of us which probably contained five atoms of iodin in its molecule. Equally negative also were the results obtained with trypan-red,



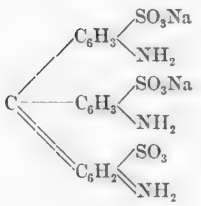
with a series of dyes of the triphenyl methane series,



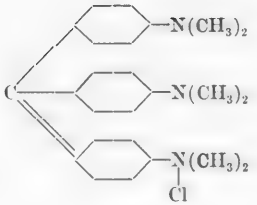
basic fuchsin,



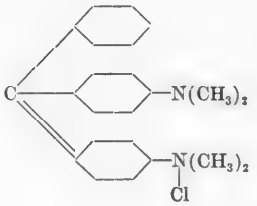
acid fuchsin,



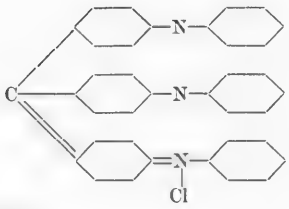
crystal violet,



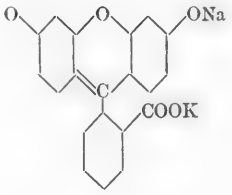
malachite green,



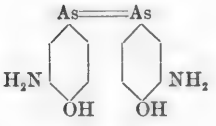
gentian violet,



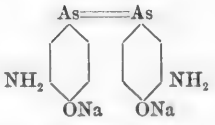
and also with erythrosin,



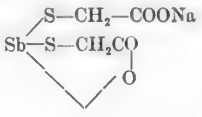
acid salvarsan,



and alkaline salvarsan,

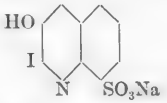


and with sodium antimony thioglycollate,

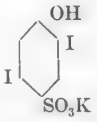


which Abel and Rowntree have shown exerted particularly marked trypanocidal powers.

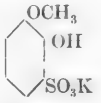
The fact that both trypan-blue and trypan-red, which are salts of sulphonic acids, are taken up selectively by the exudate led us to investigate a series of other sulphonates. Sodium iodo-o-oxyquinolin sulphonate,



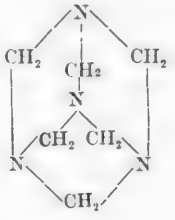
(formerly sold under the trade name of loretin or griserin) possesses this group in common with the vital stains on the one hand, and on the other hand the oxyquinolin radical in common with the quinin and cuprein derivatives used by Morgenroth, and, moreover, has been shown by Claus and his co-workers to be a strong antiseptic *in vitro* and quite harmless when injected into animals. No effect could, however, be noted from the administration of this drug, nor from potassium diiodophenolsulphonate (potassium sozoiodol),



nor potassium guaiacolsulphonate (thiocol),

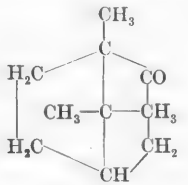


for both of which beneficial effects had been claimed in clinical therapy of the respiratory tract. Hexamethylenamin,



which Crowe and subsequent workers have shown to possess marked prophylactic and therapeutic action in various infections, was also without effect, although the milk sulphuric acid and ferric chloride test showed it to be present in large quantities in the diseased area of lung.

Seibert claims to be able to prevent death from pneumococcus septicemia in rabbits by treating them with subcutaneous injections of three to five cubic centimeters of 30 per cent. camphorated oil.



Camphor

We have tried this treatment in four rabbits, but they all died as early as the controls.

Further investigations upon the chemotherapy of experimental pneumonia are in progress.

We desire to express our thanks to Dr. Carl Glaser for advice and assistance in the preparation of iodotrypan-blue and iodoxyquinolin sulphonic acid.

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TABLE I.

Drugs and Vital Staining.

	No.	Weight. in gm.	Survival (hours).	Lobes consolidated.	Pleurisy.		Pericarditis.	Medi-astini-tis.	Remarks.
					Fibrinous.	Effu-sion.			
Control	P 1	1,550	121 (killed)	Right upper, left upper	+		Fibrinous		Culture positive.
Control	P 8		72	None					6 mg. phenolsulphonephthalein 1 hr. before death.
Control	P 14		20 (killed)	Entire left					Culture positive.
Sodium antimony thioglycollate	P 4		24	Right lower					10 mg. antimony thioglycollate intramuscularly (1 c.c. 1 per cent. solution).
Sodium antimony thioglycollate	P 4	1,070	24	Left					10 mg. sodium antimony thioglycollate 1 hr. before inoculation.
Sodium antimony thioglycollate	P 6	1,250	15	Left lung	Left	Left		+	12 mg. sodium antimony thioglycollate 1 hr. before inoculation. Blood cultures positive.
Sodium antimony thioglycollate	P 7	1,140	18						3 mg. sodium antimony thioglycollate 1 hr. before inoculation. Blood cultures positive.
Sodium antimony thioglycollate	P 10	850	14	Left lower					4 mg. sodium antimony thioglycollate 1 hr. before inoculation. Blood cultures positive.
	P 12	1,720	36	Entire left	Left		Fibrinous	+	Blood cultures positive.
Trypan-blue	P 13	1,550	14	Lower and parts of right upper					20 c.c. trypan-blue 1 per cent. solution (200 mg.) 1 hr. before inoculation. Deep blue staining of most tissues and of consolidated portions of lungs.

TABLE I.—*Continued.*

	No.	Weight in gm.	Survival (hours).	Lobes consolidated.	Pleurisy.		Pericarditis.	Mediastinitis.	Remarks.
					Fibrinous.	Effusion.			
Trypan-blue	P 5 See also B 24 and B 22	1,300	14	Entire left tip of right upper		Left (blue)			20 c.c. 1 per cent. solution trypan-blue (200 mg.) before injection. Staining as in P 13.
Iodotrypan-blue	1	Died 1 hr. after injection of iodotrypan-blue, 3 hrs. after inoculation							Died 1 hr. after injection of 5 c.c. 0.6 per cent. solution pentaiodo trypan-blue, 3 hrs. after inoculation. No vital staining of tissues or kidneys.
Iodotrypan-blue	2		18	Entire left		Bilateral	Fibrinous	+	5 c.c. 0.6 per cent. pentaiodo trypan-blue injected intravenously 3 hrs. after inoculation. No vital staining.
Trypan-red	1		36	Middle					5 c.c. 1 per cent. trypan-red intravenously 3 hrs. and 22 hrs. after inoculation. Vital staining especially of fibrin and diseased lung.
Trypan-red	2		54	Middle	Right	Bilateral			5 c.c. 1 per cent. trypan-red intravenously 3 hrs. and 22 hrs. after inoculation. Vital staining especially of fibrin and diseased lung.
Basic fuchsin (Casella)	1	Died at once							10 c.c. 0.33 per cent. fuchsin (Casella) intravenously.
Basic fuchsin (Casella)	2		18	Right lower					10 c.c. 0.33 per cent. fuchsin (Casella) subcutaneously. Slight staining of diseased area of lung. No other organs stained except subcutaneous tissue.

	No.	Weight in gm.	Survival (hours).	Lobes consolidated.	Pleurisy.		Pericarditis.	Mediastinitis.	Remarks.
					Fibrinous.	Effusion.			
Acid fuchsin (Weigert)	1		18	Right lower (not stained)		Right			10 c.c. 5 per cent. acid fuchsin intravenously. No vital staining except faint pink in tubules and pelvis of kidneys. About 10 per cent. of the fuchsin in the urine.
Acid fuchsin (Weigert)	2		70	Right middle and lower	Bilateral		Fibrinous		5 c.c. 5 per cent. acid fuchsin intravenously 3 hrs. after inoculation.
Crystal violet	2		40 min. after injection						5 c.c. 0.33 per cent. crystal violet. No vital staining except capsule of adrenal and lungs.
Crystal violet	1		Died within an hour						
Malachite green	1	Died half an hour after injection							5 c.c. 0.33 per cent. malachite green.
Malachite green	2	Died in half an hour							No vital staining.
Gentian violet	1		24	Right lower					5 c.c. 0.33 per cent. gentian violet 3 hrs. after inoculation. No vital staining; no specific staining of consolidated areas.
Gentian violet	2		36	Right lower					5 c.c. 0.33 per cent. gentian violet 3 hrs. after inoculation. No vital staining; no specific staining of consolidated areas.
Erythrosin	1		44	Right lower	Right				5 c.c. 0.33 per cent. erythrosin intravenously 3 hrs. and 22 hrs. after inoculation. No *intra vitam* staining; repeated daily.

TABLE I.—*Continued.*

	No.	Weight in gm.	Survival (hours).	Lobes consolidated.	Pleurisy.		Pericarditis.	Mediastinitis.	Remarks.
					Fibrinous.	Effusion.			
Potassium diiodophenolsulphonate (sozoiodol)	2	1,590	36	Entire left	Left		Effusion		10 c.c. 1.8 per cent. solution subcutaneously 2 hrs. before and 20 hrs. after inoculation.
Potassium diiodophenolsulphonate (sozoiodol)	1		100	Right lower					10 c.c. 1.8 per cent. solution subcutaneously 3 hrs. before and 19 hrs. after inoculation; 5 c.c. 67 hrs. after.
Sodium iodoxyquinolinsulphonate (sodium loretinate or griserin)	1		22	Right lower and middle					10 c.c. 8 per cent. sodium loretinate subcutaneously 3 hrs. after inoculation. On boiling diseased lung gives slight red color with ferric chloride.
Sodium iodoxyquinolinsulphonate (sodium loretinate or griserin)	2		24	Lower right	Bilateral		Fibrinous		10 c.c. 8 per cent. sodium loretinate subcutaneously 3 hrs. after inoculation. On boiling diseased lung gives slight red color with ferric chloride.
Acid salvarsan	1		60	Left upper	Left				5 mg. acid salvarsan intramuscularly 8 hrs. after inoculation.
Acid salvarsan			40	Middle					7.5 mg. acid salvarsan intramuscularly 8 hrs. after inoculation.
Alkaline salvarsan	1	1,800	20	Right lower and middle					25 c.c. alkaline salvarsan (25 mg.) intravenously 8 hrs. after inoculation.
Alkaline salvarsan	2		28	Lower left	Left				26.7 c.c. alkaline salvarsan (25 mg.) invenously 8 hrs. after inoculation.

	No.	Weight in gm.	Survival (hours).	Lobes consolidated.	Pleurisy.		Pericarditis.	Mediastinitis.	Remarks.
					Fibrinous.	Effusion.			
Camphor	1		36	Entire left					5 c.c. 30 per cent. camphorated oil 7 hrs. after inoculation; 5 c.c. 20 per cent. camphorated oil 36 hrs. after inoculation.
Camphor	2		24	Entire left					5 c.c. 30 per cent. camphorated oil.
Camphor	3		18	Entire left					5 c.c. 30 per cent. camphorated oil.
Camphor	4		18	Entire right					5 c.c. 30 per cent. camphorated oil.
Hexamethylenamin	1		18	Right lower	Right				5 c.c. 10 per cent. solution hexamethylenamin subcutaneously 7 hrs. later. Hexamethylenamin test in lung tissue positive and very marked.
Hexamethylenamin	2		18	Left lower					Injection as above.
Hexamethylenamin	3		12	Right and left upper	Right			+	5 c.c. 10 per cent. hexamethylenamin 2 hrs. before inoculation.
Potassium guaiacolsulphonate (thiocol)	1	1,800	36	Entire left	Left				5 c.c. 5 per cent. thiocol subcutaneously 7 hrs. after inoculation; 10 c.c. 20 hrs. and 25 hrs. after injection.
Potassium guaiacolsulphonate (thiocol)	2		18	Right upper and lower					10 c.c. 5 per cent. thiocol subcutaneously 7 hrs. after inoculation.
Benzol	B 2	3,030	24						Leucocytes at time of inoculation 640.
	B 6	1,960	22	Right multiple					Leucocytes 280.

TABLE I.—*Continued.*

	No.	Weight in gm.	Survival (hours).	Lobes consolidated.	Pleurisy.		Pericarditis.	Mediastinitis.	Remarks
					Fibrinous.	Effusion.			
	B 10	2,550	13	Entire right					Leucocytes 360.
	B 9	1,500	17	Entire right					Leucocytes 3,600.
	B 11	1,500	17	Entire left					Leucocytes 880.
	B 15	1,890	41						Leucocytes 320; temperature 40.7° C.; 6 hrs. after inoculation, leucocytes 480, temperature 42° C.; 18 hrs. after, leucocytes 180, temperature 40.7° C.
	B 18	1,790	20	Congestion and edema of left; no definite consolidation	Bilateral	Bilateral		Effusion	Leucocytes 850, temperature 39.2° C.; 7 hrs. after inoculation, leucocytes 430, temperature 38.8° C.
	B 19	1,590	20	Left upper and lower					Leucocytes 500, temperature 40° C.; 6 hrs. after, leucocytes 640, temperature 40°.
Benzol control	B 21	1,800	42	Left upper and lower	Left		Fibrinous		
Benzol control, trypan-blue	B 22	1,875	35 days	Abscess in lungs; no consolidation					Leucocytes 6,400, temperature 40° C.; 6 hrs. after, leucocytes 4,600, temperature 41° C.; 19 hrs. after, leucocytes 7,100, temperature 40.7° C.; 43 hrs. after, leucocytes 7,300, temperature 40.5°C.; on 8th day leucocytes 10,600. Very sick; 65 hrs. after, 3 c.c. 1 per cent. trypan-blue injected. Crisis (?). Respiration tubular. On 7th day urine free from chloride; chloride low until 8th day when health seemed better.

	No.	Weight in gm.	Survival (hours).	Lobes consolidated.	Pleurisy.		Pericarditis.	Mediastinitis.	Remarks.
					Fibrinous.	Effusion.			
Benzol control, trypan-blue	B 23	1,540	90	Left lower	Left		Fibrinous		Leucocytes 4,400, temperature 38.7° C.; 8 hrs. after, leucocytes 5,600, temperature 41° C.; 20 hours after, leucocytes 2,500, temperature 42° C.; 37 hrs. after, leucocytes 7,500, temperature 41.8° C.; 51 hrs. after, leucocytes 5,300, temperature 41° C.
Benzol control, trypan-blue	B 24	1,500	68 (killed)	Left lower (stained)	Left (stained)			Effusion	Leucocytes 7,500, temperature 39° C.; 7 hrs. after, leucocytes 2,100, temperature 41.2° C., 3 c.c. 1 per cent. trypan-blue (30 mg.) intravenously. 19 hrs. after, leucocytes 3,800, temperature 41.5° C.; 43 hrs. after, leucocytes 3,000, temperature 41.5° C.; 67 hrs. after, temperature 40° C.
Benzol, 2d series	B 26	2,000	Died spontaneously in cage where rabbits had been kept	Middle and purulent bronchitis					Leucocytes 700.
	B 30	1,770	16	Entire left					Leucocytes 1,000; 6 hrs. after, leucocytes 410, temperature 40° C.
	B 31	1,500	26	Right; patchy consolidation of all lobes					Leucocytes 800. Urine shows curds with silver nitrate plus nitric acid.

TABLE I.—*Continued.*

	No.	Weight in gm.	Survival (hours).	Lobes consolidated.	Pleurisy.		Pericarditis.	Mediastinitis.	Remarks.
					Fibrinous.	Effusion.			
	B 32	2,770	27	Right upper and lower (partly), and caudate			Effusion		Leucocytes 300; 11 hrs. after inoculation, leucocytes 630, temperature 41° C.; 24 hrs. after, leucocytes 750. Urine cloudy; curds.
Benzol control	B 33		7	Entire left					Leucocytes 6,500; 11 hrs. after, leucocytes 3,000, temperature 40° C.; 26 hrs. after, leucocytes 5,000; 48 hrs. after, urine gives curds; 56 hrs. after, slight clouding with silver nitrate; 72 hrs. after, clouding with silver nitrate. No curds.
	B 34		64	Entire right	Bilateral				Leucocytes 8,200; 23 hrs. after, leucocytes 10,000; temperature 39° C.; 24 hrs. after, leucocytes 4,600.
	B 35		35	Entire left; tip of right	Left				Leucocytes 4,800; 11 hrs. after, leucocytes 1,700; 26 hrs. after, leucocytes 760.
	B 36			Right lower				Effusion	Leucocytes 8,000; 11 hrs. after, leucocytes 2,600.
Toluol	T 3	2,150	Recovered						Leucocytes 5,400; 4 hrs. after, leucocytes 8,000; 28 hrs. after, leucocytes 7,400; 100 hrs. after, leucocytes 5,600.
	T 4	1,600	52						Leucocytes 5,400; 28 hrs. after, leucocytes 5,000.

ON THE COMPONENTS OF SPHINGOMYELIN.*

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The observations reported in this brief communication were made in the course of an investigation into the chemical structure of sphingomyelin. On hydrolysis of this phosphatide two substances were isolated which had not been described by previous workers. One has the nature of an acid, of the composition $C_{23}H_{47}\cdot COOH$. It was isolated as the ethyl ester, $C_{23}H_{47}\cdot COOC_2H_5$. Two isomers of the substance are known: lignoceric and carnaubic acids. The melting point of the ester seems to indicate that the isolated acid is lignoceric. The correctness of the assumption will have to be corroborated by additional evidence. The melting point of the ester, alone, and also when mixed with the corresponding ester of lignoceric acid, was 55° C.

The analysis of the substance revealed the following composition:

	Calculated for $C_{23}H_{47}.COOC_2H_5$.	Found.
C	78.80	78.50
H	13.10	13.20

The other substance had the properties of a base and was analyzed as a sulphate. It had the general properties of sphingosin, but differed in its melting point, in its solubility, and in the magnitude of its optical rotation. The substance had the composition $C_{15}H_{31}NO_2$, and hence may be regarded as a lower homologue of sphingosin. The melting point of the sulphate is 225° C. (uncorrected). The analytical data are the following:

* Received for publication, May 15, 1913.

	Calculated for $(C_{15}H_{31}NO_2)H_2SO_4$.	Found.
C	58.80	58.37
H	10.45	10.58
N	4.58	4.75
S	5.22	5.37

Most of the nitrogen of the substance can be liberated in the form of nitrogen gas by means of nitrous acid in the Van Slyke apparatus.

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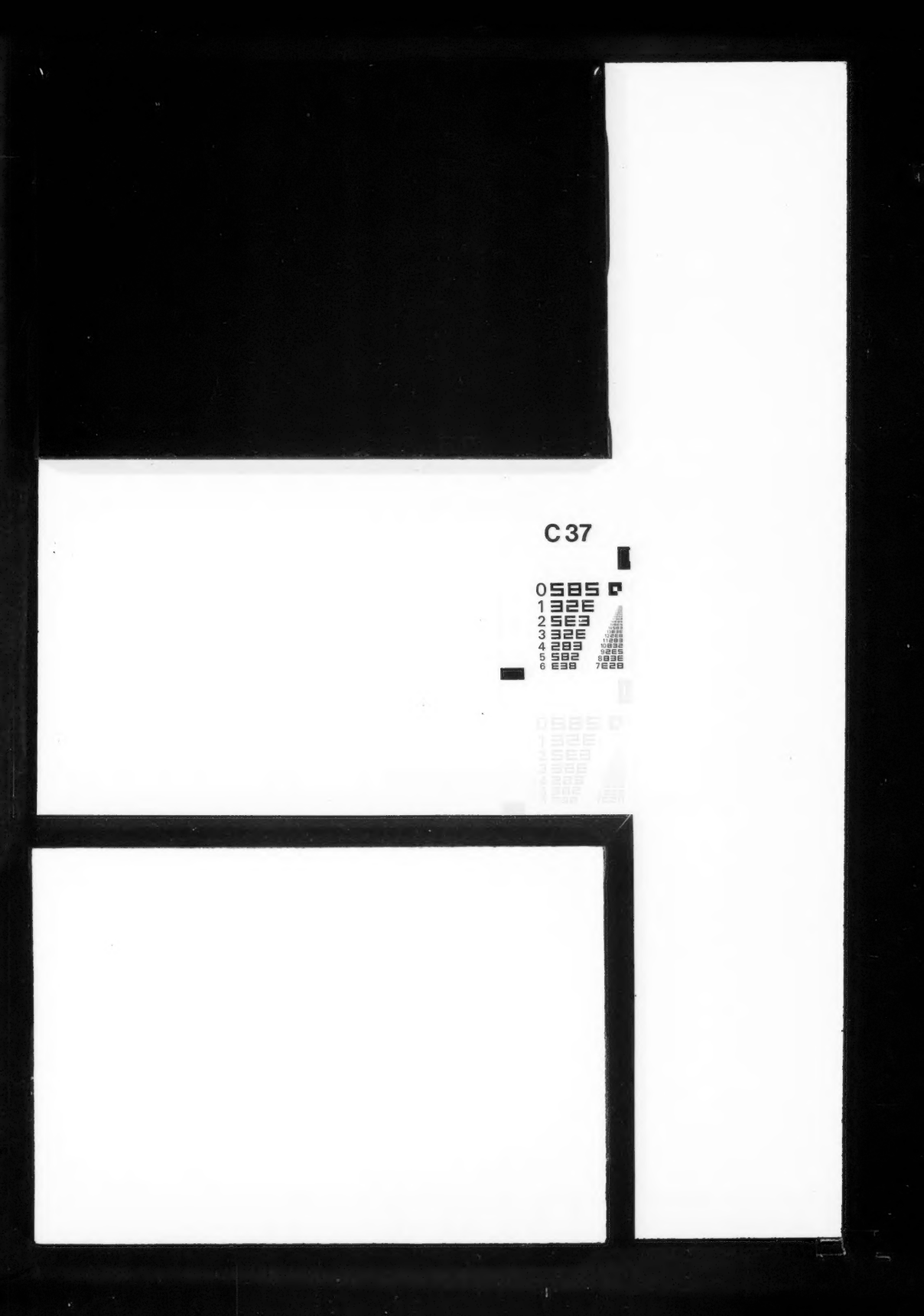
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